THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 123 SEPTEMBER 1, 1938 No. 3

THE EFFECTS OF INTRA-ARTERIAL EPINEPHRIN ON THE BLOOD FLOW IN AN EXTREMITY

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Received for publication March 31, 1938

Puccinelli (1) found that when epinephrin was introduced into the femoral artery of the dog in minimal effective doses of from 0.1 to 0.2 $\times 10^{-6}$ grams, a diminution of the outflow from the muscles ensued. Clark (2) obtained dilatation, followed by constriction, of the muscle vessels of the cat upon administering epinephrin intra-arterially in doses of about 0.05 $\times 10^{-6}$ grams. This author concluded that the site of the dilatation was the capillaries, and thought that the constrictor action was probably in the same vessels. It has also been repeatedly shown (3, 4, 5) that intravenous epinephrin produces a dilatation of the muscle vessels, as evidenced by an increase in the volume of the skinned leg, but the mechanics of this effect are obscured by the various potent actions of the drug on the heart, and on the skin and visceral vessels.

Puccinelli (6) also studied the effects on the muscle blood flow of other materials when injected intra-arterially and found that water and certain aqueous solutions had a marked dilator effect. Cori et al. (7) have recently discussed the very rapid destruction of epinephrin in dilute solution unless suitably protected, as by blood serum. These observations raise doubt as to the specificity of some of the dilator effects which have been attributed to dilute epinephrin solutions in the past when the exact method of preparation is not described and when controls showing absence of a dilator effect of the solvent are not given.

In the present experiments, the effects of small intra-arterial doses of epinephrin diluted in the recipient's whole blood were studied by means of a strohmuhr. In some experiments the limb was prepared by skinning, and in others by acute or chronic denervation.

Apparatus. The strohmuhr was a modification of the original apparatus of Ludwig (8). Its action depends upon the alternating flow of an oil piston in an

inverted U-tube, the direction of flow being controlled by a valve. Various methods have been described (9, 10) for the automatic operation of this valve, and in the present apparatus two photoelectric cells are used. Each of these is enclosed in a light-tight box and is illuminated by a beam of light focussed by a lens and passing through the oil in the inverted U-tube through a narrow slit. When this beam is interrupted by fluid (blood) rising in one limb of the tube, the direction of flow is reversed by a solenoid-operated valve. The minute currents of the photocell circuits are amplified by one stage of resistance-coupled vacuum tube amplification, the output controlling two grid-glow tubes, which in turn actuate the solenoids. The grid-glow tubes are so connected (11) that the "lighting" of one "extinguishes" the other. Each stroke of the machine is recorded on a kymograph driven by a synchronous motor (12). The accompanying diagram (fig. 1) shows the essential features of the optical and electrical arrangement. In use, the apparatus is connected by short rubber tubes to cannulae in the sectioned blood vessel in which the flow is to be measured, after an anticoagulant has been given. The kymograph

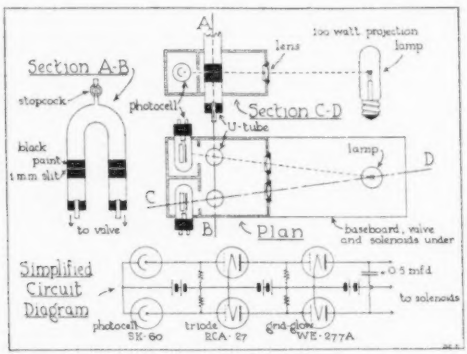

Fig. 1. Diagram of photoelectric strohmuhr. The dotted lines in the *plan* and the *section C–D* indicate the light path through the U-tube.

tracing is measured, frequently with a traveling microscope, and the flow calculated from the stroke-volume as determined at the end of each experiment.

The total error of the apparatus was studied by measurement of the actual flow at various rates and was less than 1 per cent at flows of less than 130 cc. per min. in a 15 mm. U-tube at approximately 13.5 cc. stroke-volume. At higher rates of flow, the error increases more sharply, due to distortion of the oil-water interface, and while smaller strokes give a flatter curve, irregularities between the individual measurements occur. The drop in blood pressure in the apparatus is related to the rate of flow but was less than 2 mm./Hg under the conditions described above.

Experiments. Twenty dogs of from 8.9 to 26.7 kgm., anesthetized with pentobarbital-sodium (nembutal solution, Abbott) intraperitoneally in doses of 28.5 mgm./kgm., were used. Heparin (Connaught Laboratory) was given intravenously in sufficient amounts to preclude clotting. The cannulae of the strohmuhr were inserted into the femoral artery just below the inguinal ligament. The epinephrin was in some experiments the commercial 1:1,000 adrenalin solution, and in others a 1:10,000 solution was made up from adrenalin crystals (Parke, Davis & Co.) in normal saline just before use; in either case, further dilution to $1:10^6$ was made

with a small amount of the dog's heparinized blood. The solution was injected by means of a tuberculin syringe and a small needle, which was inserted through the rubber tubing connecting the strohmuhr outlet to the artery. The blood pressure was recorded by a mercury manometer at the carotid and/or at the distal limb of the strohmuhr. In 3 animals the skin of the entire limb was removed and replaced about one-half hour before the heparin was given; in 7 animals the femoral and sciatic nerves were cut during the experiment, and in 1 animal these nerves were cut and the ipsolateral lumbar sympathetic chain extirpated 17 days previously; 190 injections of epinephrin were given, the dose varying from 0.025 to 2.0 $\times 10^{-6}$ grams, and the blood flow plotted graphically in each.

Observations. It was found that the blood flow changes could be classified into 3 types of pattern; this classification is explained in figure 2. The incidence of the various types is given in the table below; also listed (as type D) are the 33 injections which were discarded because 1, no effect could be discerned, or 2, effect was masked by rapid spontaneous fluctuations of the blood pressure and/or the blood flow.

TABLE 1

Observed incidence of epinephrin flow-patterns

TYPE OF RESPONSE	CHARACTERISTICS	NUMBER OF RESPONSES							
		After doses less than 0.18×10^{-6} grams				After doses larger than 0.18×10^{-6} grams			
		Intact leg	Denervate leg	Skinned leg	Total	Intact leg	Denervate leg	Skinned leg	Total
A	Dilatation followed by constriction	11	9	2	22	32	4	5	41
B	Constriction only	12	7	3	22	21	11	1	33
C	Constriction with superimposed dilatation	7	0	0	7	23	6	3	32
D	Miscellaneous, (unclassifiable or no definite response)	9	2	11	22	9	0	2	11

The blood pressure remained unchanged after the injections, except very rarely after a few of the largest injections (of more than 1.0×10^{-6} grams). No noteworthy difference was observed between the effects of the commercial and the crystalline adrenalin solutions when prepared as described.

No pure dilator responses are listed in the table above; pure dilatation was approached after only 4 injections, and in each of these a small constrictor phase was discernible also. The dilator phase tended to weaken, or to be masked by a greater constrictor phase, after a number of injections were given during a short period of time, as was observed by Clark (2).

All types of response occurred in the denervate and in the skinned legs, although there was an increased incidence of the type B pattern after acute denervation. This disappearance of the dilator phase after acute denervation was especially prone to occur when the nerve section resulted in an extensive dilatation. (See fig. 3.) The larger doses (more than 0.18×10^{-6} grams) produced a greater proportion of type C responses than did the smaller doses. The minimal effective dose varied in different experiments from 0.025 to 0.1×10^{-6} grams.

Discussion. It will be noted from the table that the most frequently observed flow-patterns were types A and C. These are biphasic curves produced by a combination of a slow constrictor component with a preceding or superimposed transient dilator component, the essential difference between the two types being a variation in the time relationship of the two components. To study the effect of the rate of blood flow in the

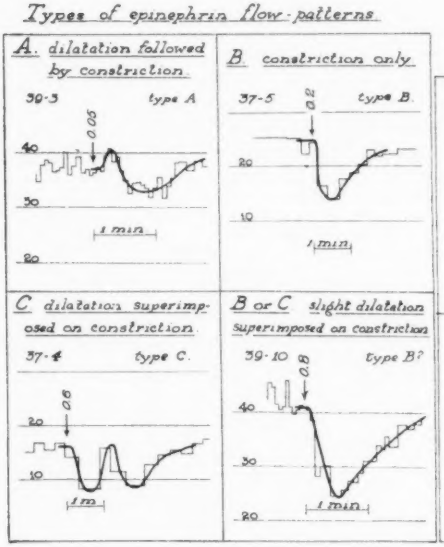

Fig. 2

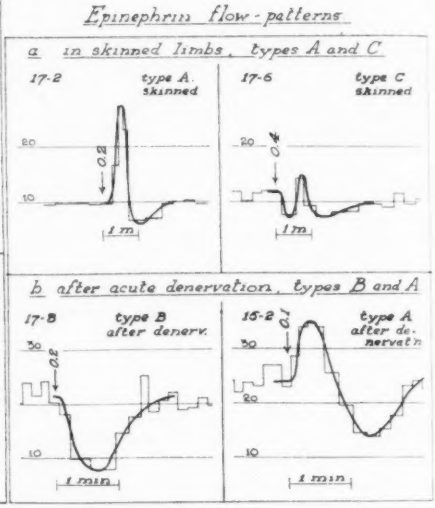

Fig. 3

Fig. 2. Classification of the flow-patterns occurring after intra-arterial epinephrin injections. The arrows indicate the time of injection, the dose being given as 10^{-6} grams. The rate of flow is given in cc./min., and time is indicated by the line below each curve, in minutes.

Fig. 3a. Flow-patterns after epinephrin in skinned limbs, graphs same scale as in figure 2. Note the persistence of well-defined constrictor phases.

Fig. 3b. Same, in acutely denervate limbs. The graph at the lower left is from the same experiment as those of figure 3a, and shows the usual absence of the dilator phase immediately after denervation. The graph at the lower right, in another animal, shows that in some cases the dilator effect may persist.

limb upon the incidence of the two types of response, the percentile graph shown in figure 4 was made. This graph shows a high degree of correlation between the rate of flow and the incidence of the two types; type A, in which the dilatation precedes any evident constriction, is the preponderant result with high rates of flow, while type C, in which the dilatation is retarded and begins after the constriction, predominates with slow flows.

Information as to the sites of the dilator and the constrictor actions of epinephrin can be deduced from this correlation. Assuming that the high degree of correlation indicates a cause-and-effect relationship, the site of the dilatation is placed distal to the site of the constriction, inasmuch as slow flows retard the onset of dilatation in relation to the constriction. Presumably, then, the dilatation occurs in the capillaries and the constriction in the arterioles. This substantiates the conclusions of Clark (2) and of Dale and Richards (3), which were largely based on analogies with the action of other drugs.

To explain the simultaneous onset of the two phases, or the occurrence of dilatation before the onset of constriction, we must then assume a small time lag in the constrictor action of the order of the time required for transport of the epinephrin to the capillaries. This lag may be the time required for penetration of the drug through the arteriolar wall, an ex-

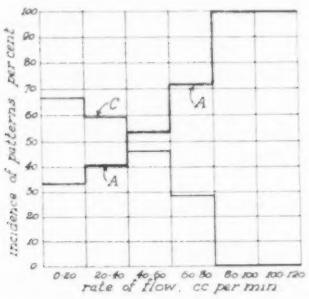

Fig. 4. Percentile graph showing the correlation between the rate of flow and the incidence of type A (cross-hatched) and type C (smooth line) patterns.

planation which is anatomically possible, because of the less intimate relationship of the muscle to the lumen in the arteriole as compared with the capillary. The flatness of the constrictor curve as compared with the sharp dilator curve also suggests a process of slow diffusion. Alternatively, it may be postulated that the arteriole has a higher threshold to epinephrin, but in the present experiments pure dilatation was not observed with the minimal effective doses.

The type of response to epinephrin thus depends upon a balance between the time required for the drug to penetrate the arteriole wall, and the time required for it to reach the capillaries; when the former time is the greater (as in rapid rates of flow), dilatation precedes constriction, and when the latter interval is greater (as in slow flows), constriction begins and dilatation is superimposed upon it. The increased incidence of type C responses with the larger doses may be explained by the greater

concentration of epinephrin penetrating the arteriole wall to its threshold more rapidly than with lesser concentrations.

Since all the curves observed were monophasic or biphasic, none suggested a triple action on two sites. Hence there is no evidence from these experiments of a capillary constrictor effect. This also seems incompatible with the well marked dilator responses obtained with many of the larger doses used.

It is thought, therefore, that the effect of epinephrin on the muscle vessels may be considered simply a combined capillary dilatation and arteriolar constriction. This concept avoids the difficulty of assuming a double effect from different concentrations of a single crystalline drug upon a single site, as is necessary if we consider epinephrin capable of producing both dilatation and, with slightly greater doses, constriction in the capillary. The constriction of the capillaries described as the result of local applications or of large doses may merely represent passive deflation secondary to arteriolar constriction, acting by reduction of the intracapillary pressure. It is also possible that transient dilator phases occurring during constriction or small preliminary constrictions as described in the present experiments may have been overlooked when the flow was measured by the venous outflow, because of the buffering action of the capillary bed and the veins.

SUMMARY AND CONCLUSIONS

Small doses of epinephrin diluted in whole blood were given in the femoral artery in dogs, and the arterial blood flow measured. The most frequent type of response was a combined dilatation and constriction, and these two phases varied in their time relationship according to the rate of flow.

This correlation indicates that the site of the dilatation is in the capillary, and that the constriction is in the artery and arteriole. No pure dilator effects were observed, though the dosage was minimal to produce a measurable effect by the method used.

It is suggested that the local effect of epinephrin upon the muscle blood vessels is, in all physiologic doses, dilatation of the capillaries and constriction of the arterioles, rather than a reversal of the capillary effect with increased concentrations.

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THE ACID-BASE BALANCE OF THE BLOOD SERUM IN HYPERTHERMIA[1]

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Received for publication April 16, 1938

It has been known for some time that fever, whether produced by various artificial means or by disease, induces a lowered bicarbonate concentration of the blood and an elevated plasma pH (Peters and Van Slyke, 1932, pp. 954, 990). Similar changes have been observed in both voluntary and involuntary hyperventilation. Since these changes are similar, it has been suggested that the effect of fever upon the acid-base balance of the blood is due to the accompanying hyperventilation (Adolph, 1923–24; Koehler, 1923; Peters and Van Slyke, 1932, pp. 954, 990). However, studies dealing with changes in the *total* acid-base balance of the blood in pure thermic fevers, as well as clinical fevers, are surprisingly lacking. Most of the observations which have been reported are confined principally to the bicarbonate concentration and the pH of the plasma. Since fever is being employed as a therapeutic procedure, it seemed desirable to make a more complete study of the changes of the acid-base balance which occur during the period of increased body temperature.

It has been known for some time that persons who are required to work in extremely hot environments usually experience less fatigue and depression if they drink water containing small amounts of sodium chloride (Moss, 1924; Talbot and Michelsen, 1933; Dill, Bock, Edwards and Kennedy, 1936). Simpson, Kislig and Sittler (1933) noted that the symptoms of exhaustion and fatigue, and the occurrence of nausea, vomiting, abdominal cramps, and muscular twitchings during sustained fever treatments can be largely overcome if the patients drink a dilute sodium chloride solution during the febrile period. As a result of these findings, it seemed of interest

[1] A preliminary report of this work was presented before the American Society of Biological Chemists, Washington, D. C., March 26, 1936. (J. Biol. Chem. **114**: xxiii, 1936.)

[2] The data presented in this paper are taken from the dissertation submitted by Wayne H. Danielson to the Graduate School of Western Reserve University, June 1936, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

to compare the changes of the acid-base balance of the blood in febrile patients who were permitted to drink a dilute saline solution with those observed in patients who were allowed to drink water only.

METHODS. Patients for this study were selected from the wards and the Out-Patient Department of the Cleveland City Hospital. Special precautions were exercised to select patients whose diseased condition would have little or no influence upon the acid-base balance of the blood. The fever was produced by means of the Kettering Hypertherm and in all of the experiments the patients' body temperature was maintained as near to 40°C. as possible during the febrile period. No drugs were administered either before or during the treatment.

The blood was withdrawn from an arm vein under oil with as little stasis as possible. It was immediately transferred under oil into a special centrifuge tube and allowed to clot. As soon as a firm clot had formed, it was loosened from the sides of the tube and the sample was centrifuged. The serum was separated from the clot at once and transferred under oil to another special tube. The serum was then analyzed according to procedures previously outlined (Muntwyler et al., 1931). Except in four of the experiments in which the blood was obtained at hourly intervals, the serum pH was determined by means of the hydrogen electrode. Inorganic phosphates were determined by the direct colorimetric method of Bell and Doisy as modified by Benedict and Theis (1924).

EXPERIMENTAL RESULTS. In order to determine the nature of the disturbances of the acid-base balance of the blood during the course of fever treatments, the blood was studied at hourly intervals on three patients who were permitted to drink water *ad libitum*. Since, on the whole, the changes were similar in each instance, the results of only one experiment are presented graphically in figure 1. The following comments can be made as a result of these experiments: 1. During the preliminary period (i.e., while the body temperature is being elevated from the normal to the febrile level) there is a marked rise in pH_s and a decrease in $(BHCO_3)_s$ coincident with a sharp fall in the pCO_2. As the $(BHCO_3)_s$ becomes decreased there is a tendency toward an increase in the $(BCl)_s$. (In the other experiments the elevation of $(BCl)_s$ was somewhat less than in the experiment presented in fig. 1.) 2. During the febrile period (i.e., while the body temperature is maintained at the febrile level) the pH_s remains increased with some tendency to return toward its pre-fever level. The $(BHCO_3)_s$ tends to fall, always reaching its lowest concentration at the end of the febrile period. Following the initial rise during the preliminary period, the (BCl) also decreases, returning toward and generally falling below the pre-fever level.

The above experiments were repeated on three patients who were permitted to drink a chilled 0.6 per cent sodium chloride solution, *ad libitum*.

This was done in order to compare the changes with those encountered when the patients drank water. The results were similar in these experiments and consequently the data of only one study are presented graphically in figure 2. It should be pointed out that the experimental data employed in the construction of figure 1 and figure 2 were obtained on the same individual. By comparing the changes, it will be observed that the essential difference lies in the curve for $(BCl)_e$. When the patient is permitted to drink a dilute salt solution, the $(BCl)_e$ does not fall during the febrile period but remains approximately at the level attained by the end of the preliminary period.

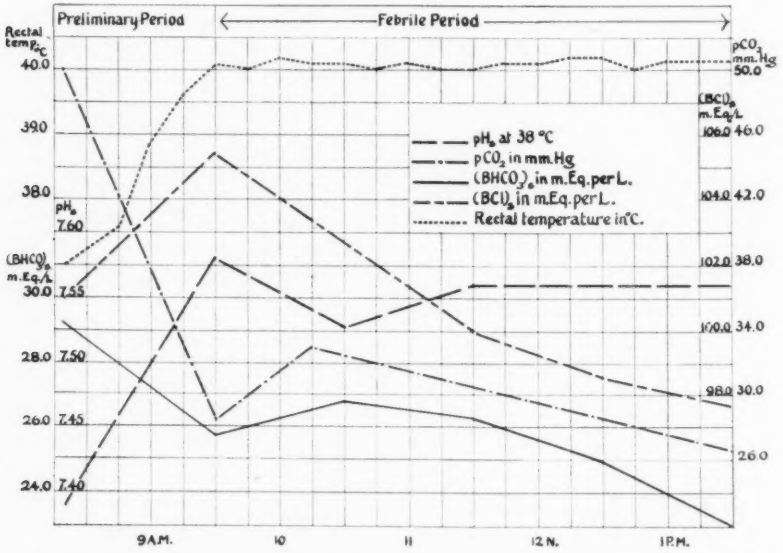

Fig. 1. The effect of hyperthermia upon the acid-base balance of the blood when the patient drank distilled water.

While there was a general uniformity in the trend of the disturbances which were encountered in the above experiments, there was some variation in the magnitude of the response. Consequently, additional experiments were performed on a series of patients in which the changes of the *total* acid-base balance of the blood were observed at the end of two and four hours of fever. As before, one group of patients was permitted to drink iced distilled water while the other group was allowed to drink a chilled 0.6 per cent sodium chloride solution. A summary of the changes which were encountered in these experiments is presented in table 1. The average control values of the acid-base constituents for the entire series of experiments are also included in the table to serve as a point of reference.

The first group of data presented in table 1 is a summary of the changes which were encountered at the end of two hours of fever (seven observations) and at the end of four hours of fever (four observations) on nine different individuals who were permitted to drink distilled water. It will be observed that in general the same type of response was obtained in all of the experiments. The only exceptions are indicated in the table with an asterisk. Thus, in one individual there was a slight increase in $(BCl)_s$ and the serum total base concentration at the end of two hours of fever while in another patient the $(BCl)_s$ was increased slightly at the end of four hours of fever. On the average, therefore, two or four hours of fever caused a

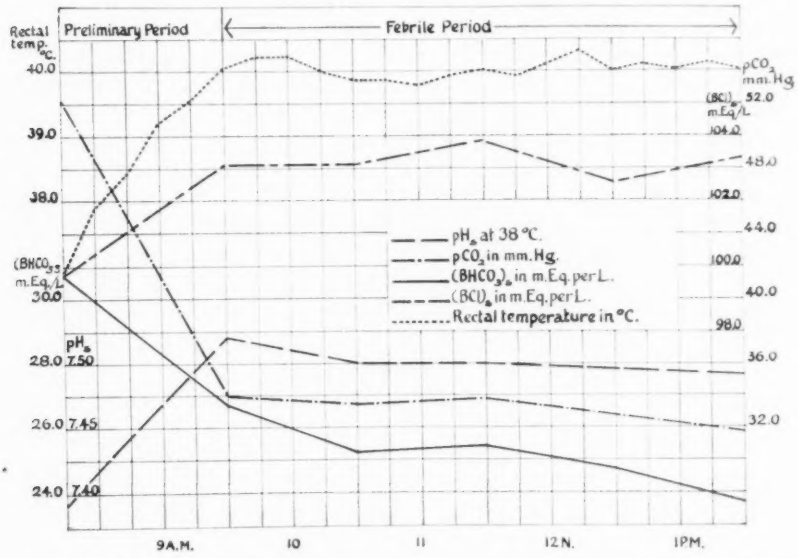

Fig. 2. The effect of hyperthermia upon the acid-base balance of the blood when the patient drank a 0.6 per cent solution of sodium chloride.

rise in pH_s, a fall in the pCO_2 and decreases in $(BHCO_3)_s$, $(BCl)_s$ inorganic phosphorus (calculated as base bound by phosphate) and the total base concentration. Due mainly to the decreases of $(BHCO_3)_s$ and $(BCl)_s$, there was a fall in the total measured acid concentration. Since the fall in the total measured acid was greater than that of the total base concentration, there was an increase in the undetermined acid fraction (presumably sulfate and organic acid). When one compares the average changes which were encountered at the end of two hours of fever with those encountered following four hours of fever, it will be noted that the pH_s was disturbed somewhat less after the longer period. All of the other constituents

listed above showed a greater deviation from the normal following the four hour treatment than following the two hour treatment.

The second group of data presented in table 1 is a summary of the changes of the *total* acid-base balance of the blood which were encountered at the end of two hours of fever (eight observations) and at the end of four hours of fever (seven observations) on ten subjects who were permitted to drink a dilute saline solution. When these data are compared with those

TABLE 1

Changes in the acid-base balance of the blood in hyperthermia

	BLOOD		SERUM									
	Oxygen content	Oxygen capacity	pH_s 38°C.	pCO_2	$BHCO_3$	BCl	BPr	BPO_4	Total measured acid	Total base	Undetermined acid	Remarks
Drinking distilled water												
	mM.	*mM.*		*mm.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	
Average	+2.0	+1.2	+0.12	−16.6	−6.0	−2.7	+2.3	−0.6	−7.9	−3.7	+4.2	2 hours of
Maximum	+3.9	+2.2	+0.24	−19.9	−9.4	−7.3	+3.9	−0.9	−12.6	−7.7	+6.2	fever in 7
Minimum	+0.3	+0.3	+0.05	−12.0	−3.0	+1.7*	+0.3	−0.3	−5.3	+0.9*	+2.3	cases
Average	+2.1	+0.5	+0.08	−18.4	−8.3	−2.9	−0.2	−1.2	−12.4	−4.2	+8.2	4 hours of
Maximum	+2.2	+0.7	+0.17	−23.6	−10.0	−4.8	−1.8	−1.2	−15.7	−7.5	+10.2	fever in 4
Minimum	+2.0	+0.3	+0.03	−15.9	−6.3	+0.6*	+2.1	−1.1	−10.3	−1.1	+6.2	cases
Drinking a solution that contained 0.6 per cent NaCl												
Average	+2.6	+0.5	+0.07	−14.5	−6.6	+2.3	+0.1	−0.9	−5.9	−4.4	+1.5	2 hours of
Maximum	+3.7	+0.8	+0.18	−18.8	−9.6	+4.4	+3.0	−1.3	−11.7	−6.8	+4.9	fever in 8
Minimum	+1.3	+0.1	0.00	−5.0	−3.1	−1.7*	−2.2	−0.6	−2.2	−1.0	−1.9	cases
Average	+3.7	+0.7	+0.08	−20.1	−9.7	+2.9	−0.6	−1.2	−8.8	−3.4	+5.5	4 hours of
Maximum	+5.0	+1.1	+0.10	−24.2	−11.4	+5.1	−2.5	−2.0	−11.0	−8.7	+8.1	fever in 7
Minimum	+0.7	+0.1	+0.04	−16.6	−7.3	+1.1	+0.5	−0.9	−6.2	+1.6**	+2.1	cases
Control values for entire series of patients studied (26 cases)												
Average	4.1	7.4	7.44	42.8	27.3	99.8	17.4	2.1	146.8	152.1	5.3	
Maximum	7.8	8.8	7.52	59.0	30.6	104.5	21.0	2.5	151.2	159.7	14.1	
Minimum	1.4	5.8	7.33	36.0	24.1	95.8	14.1	1.6	141.7	147.5	0.2	

* These exceptions are discussed in the text. In the case of the single asterisk exceptions occurred once, in that of the double asterisk twice.

in which the patient drank water it will be evident that the main difference is to be found in the serum chloride concentration. That is, instead of falling below the pre-fever level by the end of either of the fever periods, the $(BCl)_s$ remains elevated somewhat. There was only one experiment in which the $(BCl)_s$ fell below the control level. This exception is marked in the table with an asterisk. Contrary to what one might expect in the presence of an elevated $(BCl)_s$, the serum total base concentration was

found to be lowered in the majority of these experiments. There were only two of the fifteen determinations in which the total base concentration was found to be elevated slightly (indicated in table by double asterisk).

The changes of the serum total protein concentration were highly variable. Only at the end of the two hour fever treatment in which the patients were permitted to drink water was there a definite trend in the changes. At this time the serum protein concentration was elevated slightly. In all of the experiments the oxygen content and oxygen capacity of the venous blood showed some increase.

Discussion. It is apparent from a perusal of the changes presented in table 1 that the results of this study support the suggestion that the major factor responsible for the disturbance of the acid-base balance of the blood in fever is the accompanying hyperventilation. This is borne out by the lowered pCO_2, the elevated pH_s and the lowered $(BHCO_3)_s$ in all of the experiments. Observations made upon blood obtained in the hourly experiments also support this view. Thus, if the changes in the pCO_2, pH_s and $(BHCO_3)_s$ which take place during the preliminary period are plotted on triaxial coördinates as suggested by Shock and Hastings (1935), the displacement follows the hypothetical pathway for a primary carbon dioxide deficit. There is also a slight rise in the $(BCl)_s$ during this period, and in one experiment in which the changes in the total acid-base balance of the blood were determined, there is evidence of a slight fall in the serum total base concentration and a slight rise in the undetermined acid fraction. These latter changes are similar to those reported by Peters et al. (1926) in experiments of moderate involuntary hyperventilation of approximately 30 minutes' duration. After more prolonged periods of fever, the profuse sweating undoubtedly plays a part in the disturbances of the electrolytes. The large amounts of salt which are lost from the body by way of the sweat probably accounts for the steady decline in the $(BCl)_s$ noted in the water experiments. This calls attention to the experiments in which the patients drank a dilute saline solution during the course of the fever treatment. The only significant difference between the changes of the acid-base balance in these experiments and those in which the patients drank water is that the serum chloride was maintained above the pre-fever level. The drinking of the salt solution did not prevent a fall in the serum total base concentration in the majority of experiments from which one may conclude that there is a greater loss of base than chloride from the body during therapeutic fever.

In agreement with the findings of others, it has been our observation that patients experience less depression and fatigue, following fever treatments, and they appear to recover more rapidly and satisfactorily if they drink a dilute sodium chloride solution during the treatment. It was thought that the beneficial effect of the saline solution might lie in a more

rapid restoration of the acid-base balance of the blood following the febrile period. However, a series of blood studies made at the end of two hours of recovery following the two hour fever experiments revealed only slight differences in the rate of recovery following the administration of the salt. There was a definite tendency toward a recovery of the serum total base concentration but the rate of recovery of the $(BHCO_3)_s$ was somewhat slower than when the patients drank water. On the other hand, the $(BCl)_s$ remained elevated or rose to a new high. It is evident that other experiments are necessary before a definite statement can be made in explanation of the beneficial effects which the patients experience when a dilute saline solution is administered during the treatment. In this connection, it would also be desirable to have data dealing with the changes of the blood volume and water balance during fever in patients permitted to drink water and in others permitted to drink a saline solution. Such experiments are anticipated.

SUMMARY AND CONCLUSIONS

The acid-base balance of the blood serum has been studied in a series of patients who were subjected to artificial fever induced by the Kettering Hypertherm. Certain of the patients were permitted to drink a chilled 0.6 per cent sodium chloride solution during the treatment while others were allowed to drink iced distilled water only.

The results of the studies which were made at the end of the period necessary to raise the body temperature from normal to the febrile level leave little doubt that hyperventilation plays the major rôle in causing the initial disturbances of the acid-base balance of the blood in artificial fever.

In patients who were permitted to drink water only, the following blood changes were encountered at the end of either two or four hours of fever: an elevated pH_s, a decreased pCO_2 and decreases of the $(BHCO_3)_s$, $(BCl)_s$, inorganic phosphorus and the serum total base concentration. The total measured acid concentration was also decreased while the undetermined acid fraction was increased. The same changes were encountered when the patients were permitted to drink the dilute saline solution with the exception that the $(BCl)_s$ was maintained slightly above the pre-fever level.

These observations as well as the more favorable clinical condition of the patient following fever therapy when salt is contained in the drinking water are discussed briefly.

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SUSTAINED HYPERLIPEMIA OF DIETARY ORIGIN IN THE DOG

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Received for publication April 18, 1938

Numerous studies have confirmed the observation of Bloor (3) that an alimentary hyperlipemia may be produced in the dog by a single feeding of fat. Bloor (4) also demonstrated that the level of fat, phospholipids and cholesterol in the blood of the dog is increased by the continuous feeding of a diet of moderate fat content (fat = $\frac{1}{3}$ caloric value of diet) and decreased by a diet of low fat content (fat = $\frac{1}{16}$ caloric value of diet), but that the differences in levels were small in the dog in contrast to the very marked differences found under such conditions in rabbits. Bloor also found that the postabsorptive level of phospholipids, and sometimes of fat, might be elevated for two or three days after a single over-feeding to the extent of one-half the maintenance requirement in the form of carbohydrate or fat (2). In the case of phospholipids, the increase might be as high as 40 per cent.

In conjunction with McEwan and Corwin's attempts to elevate the level of cholesterol in the blood of dogs, analyses of neutral fat were made from time to time, and extremely high values for blood fats were frequently found. Because of such observations the following experiments were planned to determine, if possible, the factors involved in the production of such hyperlipemia.

EXPERIMENTAL DATA. The dogs used in this investigation were all in good condition. They were maintained on a standard diet consisting of 38 per cent ground lean horse meat, 35 per cent cracker meal, 15 per cent cooked tomatoes, 8 per cent lard and 4 per cent bone ash, which contains about three available calories for each gram of mixed diet. This was fed once daily in amounts equivalent to twice the calculated basal caloric requirement of each animal. Once each week samples of blood were taken for the determination of the serum lipids twenty-four hours after the last feeding. Additional samples of blood for the study of alimentary lipemia were taken at intervals following subsequent feeding.

After the control data had been obtained, the diet of one group of animals was changed to one feeding daily of 10 grams of lard and 4 grams of the

standard mixed diet for each kilogram of body weight. This diet represented an increase of about 25 per cent in the caloric value of the previous diet, but the nitrogen content was only slightly above the minimum. Certain animals also received daily the following additions to their diet: 1, sodium choleate, 0.25 gram per kgm. of body weight; 2, cholesterol, 0.25 gram per kgm. of body weight; 3, both sodium choleate and cholesterol; 4, phospholipid, 1.0 gram per kgm. of body weight, with sodium choleate; 5, phospholipid with cholesterol, and 6, phospholipid with choleate and cholesterol. The phospholipid used in these studies was an acetone-insoluble preparation from the adrenal glands prepared by Dr. E. C. Kendall.

Another group of animals was studied in a similar manner. These received the standard mixed diet once daily in amounts equivalent to twice the calculated caloric requirement of each animal during the preliminary and recovery periods. During the experimental period the following diets were employed: 1, the same standard diet with the addition of phospholipid, 1 gram per kgm. of body weight; 2, with the addition of phospholipid and cholesterol, 0.25 gram per kgm. of body weight; 3, 5 grams of lard and 10 grams of standard mixed diet per kilogram of body weight (this amount was approximately isocaloric with the diet of the control and recovery periods), and 4, 5 grams of lard, 10 grams of standard mixed diet, and 1 gram of phospholipid per kgm. of body weight.

Methods. *Neutral fat of the serum.* This was determined by the volumetric method of Allen, which because of the ease and rapidity with which it can be used is a highly desirable method for comparative studies. Because the cholesterol is apparently included in the column of fat, which is measured volumetrically in the Allen method, the question arises as to whether increases in neutral fat in the presence of increased cholesterol are significant. A study of the effects of artifically increasing the cholesterol by adding it in colloidal form or as a solution in butyl alcohol to the mixture of serum and fat reagent was inadequate for solution of this problem because this produced a crystalline mass in the oily layer. When the increase in cholesterol occurred in vivo, the oily layer obtained was always homogeneous.

On several occasions large samples of serum were taken and the total lipids were extracted with a 3:1 alcohol-ether mixture and petroleum ether and then weighed. Direct determinations of cholesterol and phospholipids were made and the sum of these was subtracted from the total lipid values to obtain the amount of neutral fat. A comparison of these calculated values with those obtained by the Allen method gives evidence (rough though it is because of impurities which are included in the total lipids) for the fact that increased values for neutral fat in the presence of increased cholesterol are significant (table 1).

Phospholipids. Lipoid phosphorus was determined on an alcohol-ether extract of serum that had previously been extracted with trichloracetic acid. Two cubic centimeters of serum were extracted with 8 cc. of 10 per cent trichloracetic acid for ten minutes and then centrifuged. The precipitate was washed twice with 5 cc. portions of trichloracetic acid and centrifuged. The precipitate was then extracted with 15 cc. of a 3:1 alcohol-ether mixture for an hour at room temperature, at which time it was filtered, the residue washed thoroughly with alcohol-ether, and the filtrate made up to a volume of 25 cc. Duplicate samples were ashed with 10 N sulfuric acid and superoxol, following which the phosphorus was determined by the method of Fiske and Subbarow.

Cholesterol. This was determined by the Lieberman-Burchard reaction as used by Bloor and co-workers (5).

TABLE 1

Comparison of values for neutral fat obtained (1) from total lipids by difference, and (2) by the Allen method (expressed in milligrams per cent)

TOTAL LIPIDS	CHOLESTEROL	PHOSPHOLIPID	CALCULATED NEUTRAL FAT	ALLEN NEUTRAL FAT
1—944*	213	427	304	209
2—1667*	500	687	480	447
3—926*	167	287	472	307
4—1300*	227	395	678	460
5—16970†	920	2,020	14,030	14,300
6—2660†	377	627	1,656	1,030

* Dogs.
† Geese.

RESULTS. *Neutral fats.* During the control period for the dogs maintained on the standard diet, the neutral fats of serum at the fasting level varied from 219 to 270 mgm. and averaged 254 the first week, and varied from 189 to 300 mgm. and averaged 247 the second week.

When the diet was changed to one rich in fat (10 grams of lard and 4 grams of standard diet per kilogram of body weight or 73 per cent fat) the neutral fat of the fasting serum was usually found to be less than the values found during the control period. Additions of sodium choleate or cholesterol to this diet seemed to be without effect on the fat content of the serum. The addition of phospholipid to this diet was associated with a definite increase in the fat content of the blood (table 2).

The addition of phospholipid to the standard diet was followed by a definite increase in the neutral fat content of the blood, but the further addition of cholesterol was without effect. Animals fed a diet isocaloric with the standard diet but containing 5 grams of lard and 10 grams of standard diet (36 per cent fat) per kgm. of body weight showed little change

TABLE 2

Neutral fat in blood serum of dogs receiving diets containing 73 per cent fat

DOG	WEEKS ON DIET	HOURS AFTER FEEDING							ADDITIONS TO DIET
		24	3	5	8	12	15	24	
		Milligrams per cent of neutral fat in serum							
1	9	57	105	65	52	106	117	66	
2	9	103	196	193	157	159	133	132	Sodium choleate
3	9	125	158	218	159	65	78	106	Cholesterol
4	8	508	336	438	439	440	376	509	Sodium choleate Cholesterol
5	8	738	772	847	794	720	746	866	Sodium choleate Phospholipid
6	8	1,104	1,139	1,188	1,193	1,155	1,178	1,280	Cholesterol Phospholipid
7	8	604	587	660	630	692	738	640	Sodium choleate Cholesterol Phospholipid

TABLE 3

Neutral fat content of postabsorptive serum expressed as milligrams per cent

TIME	DOG 1	DOG 2	DOG 3	DOG 4	DOG 5	DOG 6	DOG 7
	All animals receiving standard diet						
weeks							
1	240	256	219	270	260	263	277
2	189	214	252	300	260	236	280
	Diet changed to:						
	Standard + phospholipid	Standard and cholesterol + phospholipid		36 per cent fat		36 per cent fat + phospholipid	
3	438	353	432	234	196	340	466
4	388	366	486	274	214	456	516
5	428	322	391	366	128	430	652
6	482	374	632	314	202	466	626
7	349	414	395	476	270	364	627
8	468	421	523	365	248	636	798
	All animals receiving standard diet						
9	207	340	330	401	257	292	427
10	219	250	274	314	104	262	360
11	219	208	171	201	267	210	154
12	201	171	209	189	196	232	266

in the neutral fat content of the serum. The addition of lecithin to this diet produced the most marked increase in the fat content of the blood (table 3).

All of the animals which showed changes in the fat content of the serum during the experimental period returned to approximately the same level as in the preliminary studies when the standard diet was again resumed. In most of the animals normal values were reached within a week, although the lipemia persisted longer in a few.

Alimentary lipemia, as demonstrated by an increased amount of fat in the serum removed at intervals after feeding, was extremely variable. The greatest degree of alimentary lipemia was usually found in the animals receiving the standard diet. The addition of phospholipid, cholesterol, or sodium choleate to the diet was without effect on the lipemia of individual alimentation. With diets rich in fat, alimentary lipemia was frequently demonstrable during the first week of fat feeding, and occasionally marked degrees of lipemia were observed. Subsequent feedings seldom showed evidence of alimentary lipemia after the first week of the use of diets rich in fat.

Phospholipids. The serum phospholipids were found to be increased when the level of neutral fat in the serum was elevated, but the percentage increase was always less than that of the fat. It was also apparent that no direct quantitative relationship existed between the phospholipid and fat contents of the serum. Animals not receiving additional phospholipid in the diet showed an elevated plasma phospholipid when the neutral fat of the plasma was above the usual level. Animals fed phospholipid showed an increased phospholipid and fat content of blood. It should be noted, however, that all of these animals received at least 8 per cent neutral fat in their diet. When the standard diet was resumed and the neutral fat of the plasma decreased, the phospholipid of the serum also decreased but more slowly than the decrease in fat (table 4).

Cholesterol. The values for cholesterol for these dogs during this twelve-week investigation have been reported by Corwin. In general, changes in plasma cholesterol and plasma neutral fats followed a parallel course and were similar quantitatively. The addition of cholesterol to the diet was clearly without effect on the cholesterol, phospholipid, or neutral fat content of the serum.

Comment. When the fat content of the blood is increasing rapidly, as in alimentary lipemia, the plasma may become very milky. It is known, however, that even then there may be little correlation between the opacity and the actual fat content (13). It is therefore not surprising to find that when high postabsorptive lipids are produced, not by one feeding but by long-continued feedings of special diets, the plasma may be entirely clear and in no way indicate to the eye that the fat content is high.

It seems apparent that the fat content of the blood in the fasting state bears little relation to the fat content of the diet (9). After several weeks of a diet containing 73 per cent fat, the blood fat was usually less than that found with a diet containing 36 per cent fat. There was little difference in the blood fat when 36 or when only 8 per cent of the diet was fat. It is possible that our diets containing large amounts of fat were deficient in some substance other than neutral fat which influences the fat content of the blood. The addition of phospholipid to the diet in every case produced a definite elevation in the neutral fat content of blood in the

TABLE 4

Phospholipid content of postabsorptive serum, expressed as milligrams of phosphorus per 100 cc.

TIME	DOG 1	DOG 2	DOG 3	DOG 4	DOG 5	DOG 6	DOG 7
	All animals receiving standard diet						
weeks							
1	14.0	13.2	12.6	11.3	14.6		13.7
2	13.4	11.4	16.0	13.6	14.8	14.4	15.8
	Diet changed to:						
	Standard + phospholipid	Standard and cholesterol + phospholipid		36 per cent fat		36 per cent fat + phospholipid	
3	15.3	17.1	19.6	15.8	12.8	18.4	18.6
4	17.3	15.0	17.2	15.2	12.6	19.7	18.7
5	17.8	19.4	19.8	20.3	13.9	17.2	22.7
6	18.8	13.6	15.1	21.7	20.8	18.7	15.6
7	26.8	13.2	12.6	23.9	14.3	22.2	21.9
8	27.8	11.4	16.0	20.0	16.5	25.2	24.8
	All animals receiving standard diet						
9	16.4	17.1	19.6	21.7	16.0	15.0	19.8
10	15.1	15.0	17.2	17.9	11.4	18.7	21.5
12	12.5	13.6	15.1	17.9	13.5	16.8	18.4

fasting state. The phospholipid of the blood was also increased, but the relationship between the neutral fat and phospholipid in the blood was obviously not quantitative. When phospholipid was withdrawn from the diet the blood fat usually decreased more rapidly than did the blood phospholipid.

Eichholtz, in 1924, fed 40 grams of lecithin to young dogs and found slight increases in blood lecithin in two, four and six hours, but no appreciable change in neutral fat. Sueyoshi and Okanogi found an increase of lipoid phosphorus in corpuscles and plasma of rabbits which were given

a single injection of lecithin, with the maximal increase twenty-four hours after the injection. Pasternak and Page injected cephalin intravenously into rabbits in amounts varying from 0.1 to 1.5 gram per 1 kgm. and found increases in blood fats which decreased rapidly at first and then more slowly, so that it often took one day for their return to normal. The phosphatide excess disappeared more rapidly than that of the other lipoids. With repeated injections of small doses of cephalin over a period of a month, the blood plasma remained normal. Rewald, earlier had fed two dogs lecithin for six and fifteen months, respectively, and found the blood lipoid phosphorus higher than normal.

Kaplan and Chaikoff found a heat-labile factor in raw pancreas which, upon ingestion, will produce a rise above normal in all the lipid constituents of the blood of depancreatized dogs maintained with insulin, particularly cholesterol. Choline, in daily amounts of 3 grams for three weeks, did not produce levels above normal in these animals. No observations on the possible effect of raw pancreas on the blood lipids of normal dogs were recorded.

The phospholipid preparation from the adrenal glands which was used in our experiments, and which apparently produced very marked hyperlipemia as used, was not a highly purified preparation. This experiment will therefore be repeated using commercial lecithin. That this also may produce a hyperlipemia is suggested by earlier experiments of McEwan in which he obtained a similar hyperlipemia in dogs which were fed, daily, six eggs (approximate lecithin content 12 grams) (15) along with a diet rich in fat.

SUMMARY

A sustained postabsorptive hyperlipemia may be produced in dogs by feeding them phospholipids daily with a diet consisting of 8 per cent, 36 per cent, or 73 per cent fat. A somewhat greater lipemia is observed with diets richer in neutral fat. The increase in blood lipoid is largely neutral fat and cholesterol, although the phospholipids are also increased.

The blood lipoids are not greatly altered by increased amounts of neutral fat or cholesterol in the diet.

The hyperlipemia quickly subsides when phospholipids are omitted from the diet.

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OBSERVATIONS ON CREATININE AND UREA CLEARANCES, ON RESPONSES TO WATER INGESTION AND ON CONCENTRATING POWER OF KIDNEYS IN NORMAL, DIABETES INSIPIDUS AND HYPOPHYSECTOMIZED DOGS

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Received for publication April 18, 1938

This paper reports on investigations of the water exchange and renal function of adult dogs *a*, in the normal state; *b*, during polyuria produced by surgical procedures on the hypothalamo-hypophyseal system; *c*, after hypophysectomy (removal of pars anterior, pars intermedia, infundibular process and part of stalk), and *d*, under influence of anterior lobe extract. Observations have been made on daily urine output, on acute responses to large water ingestion, on urea and creatinine clearances and on maximum concentrating power with water deprivation. Normal controls were first obtained, then the same animals subjected to operation. Fifty-one dogs have been used; among these are fifteen on which we have preoperative and postoperative data other than daily urine output. For all experiments involving urine collections other than 24 hour samples we used females previously prepared by midline perineotomy.

METHODS. The dogs were kept continuously in metabolism cages except for the occasional brief periods of experimentation. The amount of food (cooked horse meat plus Purina dog chow) for each dog required to maintain constant weight was soon established and this amount and composition kept constant. Each dog was observed in the normal state for 1 to 4 months. During this time, in addition to following daily urine output, determinations of clearances and of response to water ingestion and deprivation were made. Operation was then performed and such observations repeated.

Early in this work the hypophysis was approached by the temporal route, later through the palate. With the latter approach the undisturbed gland and its rostral environs are clearly seen and the one can be attacked with a minimum of trauma to the other. Although the operative field is necessarily not sterile, infections are surprisingly few. The dogs eat and drink normally within a few hours after such operations. Where indicated, the nature of the lesions was established by serial sectioning

from the chiasma to the mammillary bodies. The details of the anatomical findings and their correlation with daily urine output will be reported elsewhere. It will suffice here to say that the permanent polyurias of this paper were produced by lesions between the chiasma and the hypophyseal stalk, with destruction of the median eminence (ME) in some cases, and that with an appropriate lesion the presence of anterior lobe is not essential to a maximum and permanent polyuria.

The water ingestion experiments were as follows. In the postabsorptive state urine was collected for 4 to 6 control periods of 15 minutes, then 3 per cent body weight of water was given by stomach tube and the collections continued for 12 to 16 more periods. Blood was drawn from an ankle or jugular vein and serum conductivity determined as an index of salt dilution (White and Findley, 1937). In both these and the clearance experiments the dogs must be well trained and undisturbed. Fright almost always produces a sharp fall in a diuresis curve, usually recovered from in 30 to 45 minutes. There is about a 50 per cent probability that creatinine clearances will fall on fright; cases have been observed where urine output fell with no change in creatinine clearance.

Clearances were carried out as follows. In the postabsorptive state creatinine (0.2 gram per kilo) was given in 2 to 3 per cent body weight of water by stomach tube. Beginning 75 to 90 minutes later three consecutive 30 minute urine collections were made, with a blood sample at the middle of each period. Creatinine determinations on serum and urine were essentially according to Folin. Usually urea clearances were determined at the same time but sometimes independently. Serum urea was determined by permitting urease action, followed by a zinc-barium precipitation recommended by Somogyi, with direct Nesslerization of the filtrate. Preformed ammonia was removed from urines by permutit; this was followed by urease action and Nesslerization. The analytical errors, as shown by occasional duplicates, by analyses of known creatinine and urea solutions and by recoveries from serum and urine, did not exceed $\pm$ 3 per cent and were usually less than 2 per cent.

In the water deprivation experiments urine creatinine and urea were determined as above, chloride by a Volhard titration.

Results. *Daily urine output.* It is not the purpose of this paper to consider in detail the correlation between nature of lesion and effect on daily urine output. Section of the stalk or hypophysectomy, if done without disturbing the region of origin of the stalk, causes only a very brief (24 hour) or no polyuria. High (5 to 10 times control output) and permanent polyurias were produced by postchiasmal lesions interrupting the supraoptic tracts (as evidenced by degeneration of supraoptic nuclei (see figs. 1 and 2)) and destroying the ME at the origin of the stalk. Whether the latter step adds anything to the effect of supraoptic tract

interruption we are not yet prepared to say. Given such lesions, we now believe that the presence of the anterior lobe is not essential to the full polyuria.

On numerous occasions in this series we have produced a polyuria (4 to 6 times normal output) of 3 to 5 days' duration, by giving intraperitoneally on one or on two consecutive days 0.5 gram per kilo of fresh beef anterior lobe in a saline suspension or an acid extract of 0.1 gram per kilo of beef anterior lobe powder, in normal and in hypophysectomized dogs. On one occasion we had the strange experience of seeing such a two-day period of anterior lobe administration restore permanently to a high level a previously high polyuria, due to hypothalamic puncture, which had slowly declined to about $2\frac{1}{2}$ times the control output. This high level

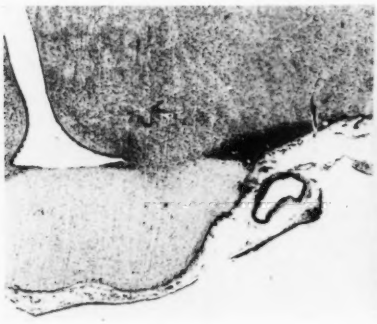


Fig. 1

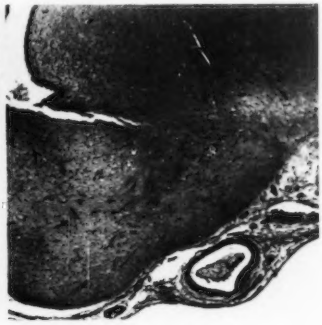


Fig. 2

Fig. 1. Frontal section through normal dog's optic chiasma showing supraoptic nucleus.

Fig. 2. Frontal section through chiasma of DI dog subjected to interruption of supraoptic-hypophyseal tracts 88 days before death, showing atrophy of supraoptic nucleus.

(10 or more times normal, i.e., 5 to 7 liters daily) persisted, with a late fall (to about 3 liters daily), for $4\frac{1}{2}$ months, when the experiment was terminated. On two other occasions attempts to restore declining puncture polyurias by the same means were unsuccessful.

Response to water administration. The response to administration by stomach tube of 3 per cent of body weight of water has been followed in 28 experiments on 13 normal dogs, in 14 experiments on 5 diabetes insipidus (DI) dogs and in 14 experiments on 3 hypophysectomized dogs without polyuria. Only a few salient features of this accumulation of results can be discussed here. It will be remembered that every operated dog had first been subjected to observation while normal.

The water response results may perhaps best be first considered by

examining the detailed findings of one typical experiment each on a DI and on a hypophysectomized dog and comparing them with the preoperative findings with the same procedure on these same dogs.

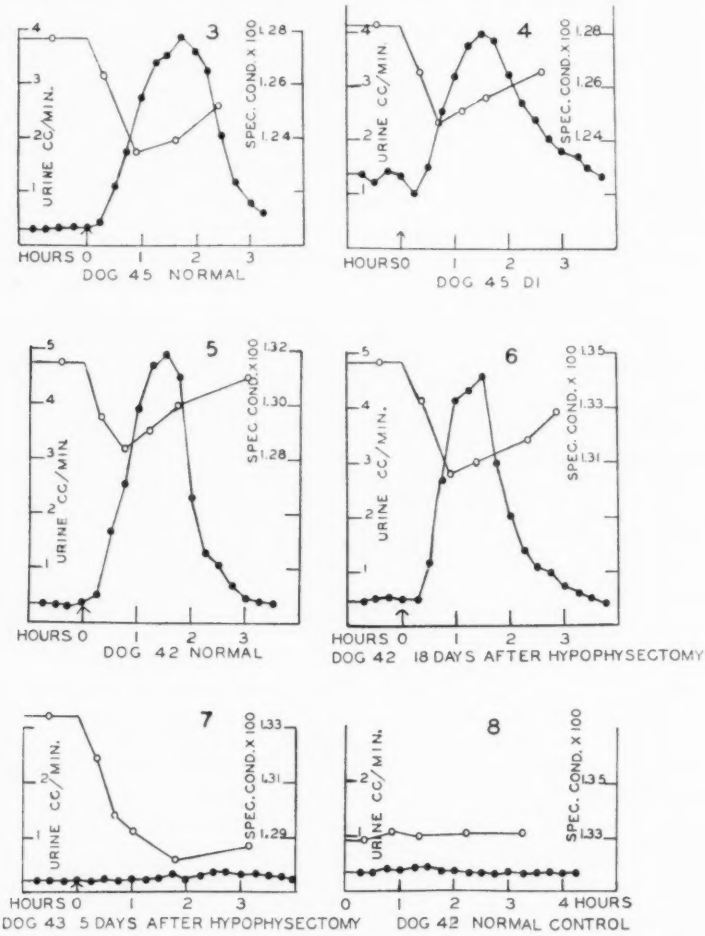
A comparison of figures 3 and 4 shows that the lag of maximum rate of water output behind maximum mol fraction of water in serum (conductivity minimum) was no less in dog 45 while she was DI (daily output 7 to 10 × normal) than while normal. The same is found for hypophysectomized dog 42 (figs. 5 and 6). These findings extend those of Newton and Smirk (1934) on acutely hypophysectomized preparations and speak against the view of Verney and collaborators (1933) that the lag in water output behind water load represents the time for preformed pitressin to disappear, since in our operated animals there was presumably little or no preformed pitressin. The blood findings show that a normal interval between water administration and crest of urine output in DI and hypophysectomized dogs cannot be ascribed to a delay in blood dilution.

The average of 28 experiments on 13 normal dogs shows that maximum rate of urine output was attained at 104 minutes after the water administration. The average of 11 experiments on the 5 normals (dogs 16, 17, 37, 44 and 45) subsequently made DI by hypothalamic puncture shows the urine output crest at 102 minutes; the average of 14 experiments on these 5 dogs when they were DI shows a crest at 95 minutes. The average of 10 experiments on 3 normal dogs subsequently hypophysectomized shows the crest at 105 minutes; the average of 14 experiments on these 3 dogs after hypophysectomy shows the crest at 110 minutes. Blood samples were not taken in all of the experiments.

Within the first week or 10 days after hypophysectomy dogs may show little or no diuretic response to 3 per cent body weight of water by mouth; by 2 weeks postoperative and thereafter they show diuresis curves normal as to time relations but usually lower than normal. The 14 posthypophysectomy experiments of the preceding paragraph were performed at 2 weeks or more after operation. Figure 7 shows a typical response early after hypophysectomy (dog 43); one dog, however, (dog 42) showed a diuresis curve of practically normal height 8 days after hypophysectomy as well as thereafter. Figure 8 shows a control experiment, i.e., urine output and serum conductivity on normal dog 42 over a 4 hour period with no water administration.

Where diuresis is absent or scanty, either with early hypophysectomized dogs or with untrained normal dogs, blood dilution is greater and lasts longer than when diuresis is normal. Such a longer duration of dilution does not indicate a delay in water absorption but merely a failure to get rid of the water. It is concluded that the time relations of the response to a large dose of water are not significantly altered in the DI or the chronically hypophysectomized dog.

In both hypophysectomized and DI dogs the total electrolyte content of the serum, as indicated by conductivity, is not consistently raised or



Figs. 3 to 8. Serum electrolyte dilution and urine output responses of dogs to 3 per cent body weight of water; serum specific conductivity in reciprocal ohms; urine output in cubic centimeters per minute. Three per cent body weight of water by stomach tube at zero hour except in figure 8, which shows a control, i.e., normal dog with no water administration.

lowered from the normal but shows a wider range of spontaneous fluctuations.

CLEARANCES. *Diabetes insipidus.* The typical behavior of the creatinine and urea clearances after development of DI may be seen in figure 9 (dog 16), which also shows the effect of an anterior lobe injection. In

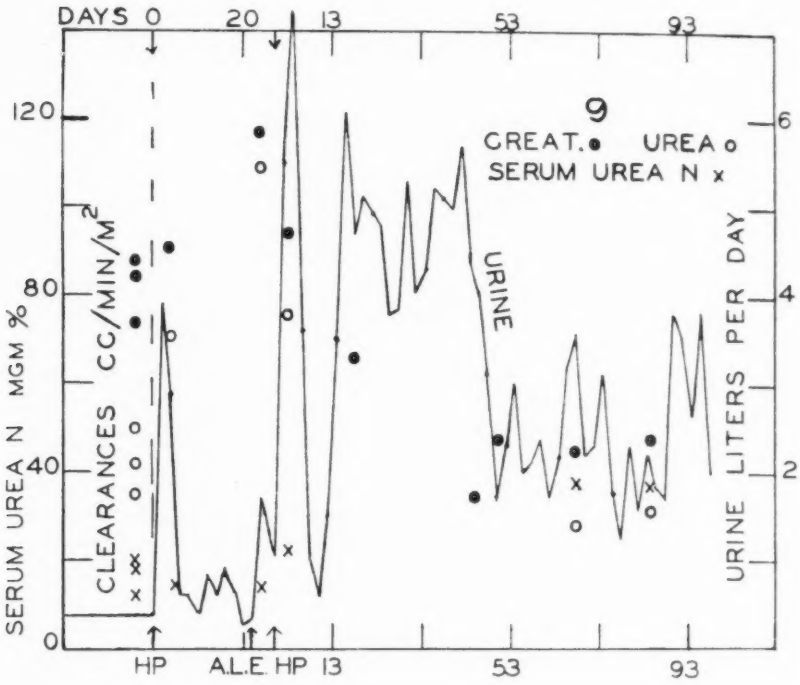


Fig. 9. Dog 16. Daily urine output in averages of 2 day periods, creatinine and urea clearances and serum urea N before and after hypothalamic puncture, *HP*, resulting in diabetes insipidus. Urine output to left of first *HP* is average of a 53 day preoperative period (395 cc. daily). First *HP* results in only transitory polyuria. Anterior lobe extract (0.5 gram fresh beef gland per kilo) intraperitoneally for 1 day at *ALE*. Second *HP* produces transitory phase, normal interphase and permanent polyuria. Horizontal axis expresses time quantitatively after first *HP* but not before. Numbered days begin with second *HP*. Each clearance entry in this figure, as well as in figures 10 and 11, represents the average of 3 consecutive 30 minute period clearance determinations.

general, creatinine clearance is unchanged during the transitory polyuria. Urea clearance may be somewhat elevated but this is probably because of greater urine flow. Although we gave water in the clearance experiments with the purpose of keeping urine flow about the same during the clearance

periods with all dogs, the flow has occasionally been lower during a clearance determination in the normal or hypophysectomized dogs.[1]

Creatinine and urea clearances during the "normal interphase" between the transitory and permanent polyurias are normal. These clearances remain normal for 1 to 2 weeks after the onset of the permanent polyuria and then both gradually fall, creatinine reaching a level approximately half the normal by 4 to 6 weeks after the operation. Urea clearances fall less than creatinine and may not fall at all even with a 50 per cent fall in creatinine; this may result in an unusually high ratio of urea to creatinine clearance values. This situation is complicated by the fact that, on the average, the urine flows during the clearance periods of the permanent polyuria were somewhat higher than during normalcy or latent period. This would work in the direction of raising urea clearances. A slight fall or no change under our conditions might have been a more definite fall if urine flow rates could have been kept constant during all clearance periods. The values have returned not quite but almost to normal after 175 days of permanent polyuria. The above statements are based on 29 creatinine and 22 urea clearance values made on 5 dogs (16, 17, 37, 44 and 45) preoperatively and at various times after production of a lesion which resulted in permanent DI, where each clearance value is the average of 3 consecutive 30 minute clearance periods. These data are presented in figure 10.

In addition to the above, 15 creatinine and 13 urea clearance values have been obtained on 4 normal dogs without postoperative determinations. The average values for these 4 dogs are 94 cc. per minute per square meter for creatinine clearance and 53 for urea.

Hypophysectomy. Similar observations have been made on 3 dogs before and after hypophysectomy (dogs 38, 42 and 43). The data are presented in figure 11. It is seen that both clearances fall after hypophysectomy and are returning toward normal at the end of a few weeks. In no case has albumin or glucose been present in the urine of our DI or hypophysectomized dogs.

Anterior lobe administration. The effect of anterior lobe administration on creatinine and urea clearances has been investigated in 2 normal dogs. In both animals intraperitoneal administration of extract equivalent to 0.5 gram fresh beef anterior lobe per kilo resulted in a several-fold increase in daily urine output, a rise of 25 to 35 per cent in creatinine clearance and

[1] In most periods with all dogs flows have been between 1 and 4 cc. per minute; most dogs show but little change in urea clearance within these limits of urine flow. We agree with Shannon (1936) that in general there is a tendency for urea clearance to increase progressively with rate of urine flow even above 2 cc. per minute, but with most dogs the increase in clearance above 1 cc. per minute is not great, i.e., at rates between 1 and 5 cc.

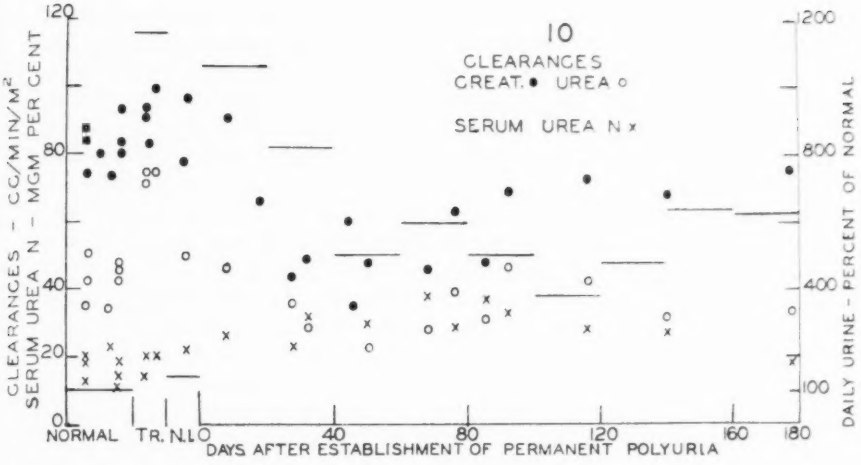


Fig. 10. Daily urine output in averages of 20 day periods, creatinine and urea clearances and serum urea N before and after establishment of polyuria. This figure shows findings on 5 DI dogs. Urine outputs are expressed as percentages of the normal, averaged for 20 day periods in all 5 dogs, and shown as the horizontal lines. Left section of figure, labelled *Normal*, shows urine output (100 per cent) and clearances during the preoperative observation periods of 24 to 53 days with the various dogs. Transitory phase of polyuria, *Tr.* and normal interphase, *N.I.* Horizontal axis expresses time quantitatively after zero day.

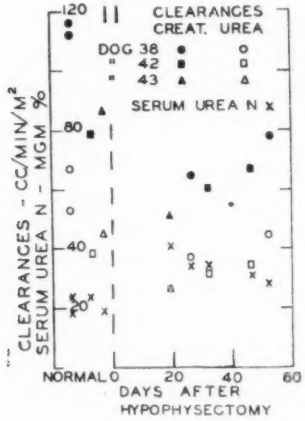


Fig. 11. Creatinine and urea clearances and serum urea N on 3 dogs before and after hypophysectomy.

a rise of 60 to 80 per cent in urea clearance; the urea–creatinine clearance ratio was raised from the normal of about 0.55 to 0.80 or 0.90. The influence of anterior lobe apparently both raises rate of glomerular filtration and diminishes the extent of reabsorption of urea; serum urea is unchanged or slightly lowered.

Maximum concentrating power of kidney. Urine concentration after 24 hours of water deprivation was observed in 3 DI and in 3 hypophysectomized dogs, preoperatively and postoperatively. In these experiments water was removed for 24 hours but the usual food given. After 24 hours of water deprivation the animals' bladders were emptied and the urine formed during the next 3 or 4 hours collected by a second catheterization. Neither the normal nor the hypophysectomized dog showed any greater urine concentration after 48 than after 24 hours of water deprivation. The averages of the findings are shown in table 1. It is seen that in both conditions there is a marked impairment, as compared with the normal, of ability to concentrate urea, with a somewhat less markedly deficient

TABLE 1

Average concentration of urine after 24 hours of water deprivation

	SPECIFIC GRAVITY	CREATININE	UREA N	NaCl
		mgm. per cent	*mgm. per cent*	*mgm. per cent*
Normal preoperative	1.068	190	4,270	1,100
Postoperative DI	1.035	145	1,670	860
Normal preoperative	1.064	220	4,100	1,050
Postoperative hypophysectomized	1.040	160	2,100	1,020

ability to concentrate creatinine; concentrating power toward chloride is not greatly altered in either condition. On the other hand, the DI animal is capable of putting out a much more concentrated urine than it habitually does when given free access to water. The hypophysectomized animals show no obvious diminution in tolerance to water deprivation, as evidenced by the fact that they as well as the normals show no signs of distress other than thirst after 48 hours of water deprivation. The DI animals, on the other hand, can hardly tolerate more than 28 hours of deprivation. On one occasion one DI dog was deprived of water 48 hours, when she was found unconscious. She appeared moribund for several hours, but recovered after water and salt solution by stomach tube and intravenous injection of glucose solution.

Miscellaneous. The response of 2 of our DI animals to pitressin was tested. With 2 subcutaneous injections of 5 units of pitressin 10 hours apart the urine output for the corresponding 24 hour period dropped almost to normal but was up the next day and thereafter.

We have been impressed with the finding that occasionally dogs showing consistently high 24 hour urine outputs will show normal rates of flow for periods of a few hours. Thus, normal dogs of 7 to 18 kilos under our basal conditions (out of cage for 2 hours without water and in postabsorptive state) show urine flows of 0.2 to 0.5 cc. per minute. A dog showing such a flow while normal may while DI show 1.5 to 4 cc. per minute without other than voluntary water intake but will at times show for several consecutive 15 minute periods rates within the normal range, even on days when the 24 hour output is 5 to 10 times the normal, i.e., such that the average flow per minute for the 24 hour period is 2 to 4 cc. We have no explanation for these occasional lapses to normal rate; they have been observed without any associated fright or exercise.

Longer dips in the urine curve are also frequently seen. Thus, a dog may show a high 24 hour output for many days, then a period of one to several days of a much lower output, perhaps even within the normal range; this followed by a return to a high level. Several such cycles may be seen. These changes are not associated with any change in food intake or any other factor which we can identify. Some dogs do not show such cycles. We can only conclude that some unknown factors are at work; it is permissible to guess that residual fragments of pitressin-forming tissue may be able to function intermittently.

SUMMARY

Hypophysectomized or DI dogs, in which exists a more or less complete chronic deficit of pitressin, show no significant alteration from the normal in the time relations of their blood dilution and urine output responses to ingestion of 3 per cent body weight of water. This is interpreted as indicating that the lag of maximum rate of water output behind maximum blood dilution after water drinking in the normal is not due to the time required for preformed pitressin to disappear. Within the first week or ten days after hypophysectomy there may be only a slight and delayed diuretic response to water by mouth.

Creatinine clearance is unchanged during the transitory phase of experimental DI, urea clearance somewhat elevated probably because of increased urine flow. Both clearances are normal during the normal interphase. Soon after onset of permanent polyuria both clearances gradually fall. Creatinine clearance reaches a level approximately half the normal by 2 to 4 weeks after onset of permanent polyuria, remains down for a variable time and then usually returns towards normal, which may be almost reached after 6 months of permanent polyuria. Urea clearance falls less than does creatinine during permanent polyuria and may not fall at all, with the result that the urea/creatinine clearance ratio

may be as high as 0.8. Serum urea rises as clearances fall early in permanent polyuria but shows a later return toward normal.

Under the influence of anterior lobe administration creatinine clearance rises slightly, urea clearance to a greater extent, the urea/creatinine clearance ratio rising to 0.8 or 0.9. Serum urea is unchanged or slightly lowered.

After 24 hours of water deprivation the ability of hypophysectomized (without polyuria) and of DI dogs to concentrate urine is impaired about 50 per cent with respect to urea, 25 per cent with respect to creatinine and unchanged with respect to chloride. The ability of hypophysectomized dogs to tolerate water deprivation is as great as that of the normal, but that of DI dogs is greatly decreased.

Dogs with permanent polyuria may show temporary phases of normal rates of urine output.

This work was aided by a grant from the Committee on Scientific Research of the American Medical Association.

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INFLUENCE OF THE THYROID GLAND ON ABSORPTION IN THE DIGESTIVE TRACT[1]

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Received for publication April 19, 1938

The present investigation was undertaken in order to ascertain the influence of the thyroid hormone on absorption in the alimentary tract. Our interest in this subject was aroused when, in attempting to obtain information on the nature of lowered "tolerance" to dextrose in hyperthyroidism, we found that oral administration of galactose also resulted in abnormally high curves for this sugar in the blood of patients with this disease (1). Since utilization of galactose was found to be normal in hyperthyroid patients (2), it was considered desirable to determine experimentally whether increased absorption of sugars accounted for this phenomenon. When increased absorption of sugars was found in hyperthyroid rats, further experiments were performed in order to discover the mechanism by which thyroxin increases absorption.

EXPERIMENTS. In order to clear the intestine of food residues, young rats were fasted in a metabolic cage for 24 hours before performing the absorption experiments. They were then given by stomach-tube measured amounts of the substance under investigation. In each instance the amount was calculated to exceed the capacity for absorption. After a given period of time, the animals were sacrificed in order to determine the amount of unabsorbed residue in the digestive tract. In most of the experiments the contents of the entire digestive tract were used for determinations; in two experiments (tables 3 and 5) the gastric and intestinal contents were analyzed separately.

This procedure was carried out on normal, on hyperthyroid and in two experiments on hypothyroid rats. Rats were rendered hyperthyroid by intraperitoneal injections of 0.1 mgm. of thyroxin per 100 grams of body weight, given daily for 12 days. The average basal metabolic rate of rats so treated, as determined in a closed circuit apparatus similar to that used by Benedict and Macleod (3), is increased about 50 per cent. To

[1] Aided by a grant from the Christine Breon Fund for Medical Research.

Read in part at the meeting of the American Society for Clinical Investigation, Atlantic City, May 3, 1937.

render rats hypothyroid, the thyroid gland was excised under ether anesthesia three weeks prior to the absorption experiment. The basal metabolic rates of these animals averaged 43 per cent minus.

The original body weight was chosen as a basis for calculation of the rate of absorption of a given substance per unit of body weight because both administration of thyroxin and thyroidectomy cause loss of weight without reducing the absorptive area of the gut.

Female rats were used for all experiments except as indicated in table 1.

The absorption of sugars. Dextrose, galactose and xylose were administered in the form of a 20 per cent solution in all but one of the experiments. This concentration was chosen to insure the maximal rate of absorption,

TABLE 1

Absorption of dextrose, galactose and xylose from the digestive tract in normal, hyperthyroid and thyroidectomized rats

EXPERIMENTAL CONDITION	SEX	NUMBER OF RATS	RANGE OF ORIGINAL WEIGHT	LOSS OF WEIGHT AFTER FASTING 24 HOURS	SUGAR USED	AMOUNT OF SUGAR FED PER 100 GRAMS OF ORIGINAL WEIGHT	AMOUNT OF SUGAR ABSORBED PER 100 GRAMS OF ORIGINAL WEIGHT
			grams	*per cent*		*mgm.*	*mgm.*
Normal	M	6	174–230	5.2	Dextrose	477	129 ±16*
Hyperthyroid	M	7	180–240	12.2	Dextrose	454	252 ±27
Thyroidectomized	M	7	175–246	13.1	Dextrose	404	78 ±19
Normal	F	8	170–215	7.9	Dextrose	379	173 ±9
Hyperthyroid	F	8	150–210	18.8	Dextrose	377	284 ±30
Thyroidectomized	F	4	148–165	12.9	Dextrose	339	91 ±5
Normal	M	4	226–320	4.4	Galactose	360	192 ±27
Hyperthyroid	M	8	225–276	17.7	Galactose	414	306 ±49
Normal	F	8	130–192	6.8	Xylose	279	31 ±3
Hyperthyroid	F	5	130–170	19.0	Xylose	261	53 ±9

* Standard deviation.

which is induced when solutions containing 10 per cent or more of dextrose are administered (4). At the end of an hour, quantitative determinations of sugar in the stomach and intestine were performed by the method of Hanes (5). In one experiment, a 5.4 per cent solution of dextrose was given. In this instance it became necessary to terminate the experiment at the end of 30 minutes because otherwise the amount of solution needed to determine the rate of absorption would have exceeded the capacity of the stomach.

Figures for absorption by *normal rats* of the various sugars are given in table 1. In this table it is seen that the male rats absorbed 25 per cent less dextrose than the females.

Among the *hyperthyroid rats*, the males and females absorbed respectively 95 and 64 per cent more dextrose than the normal controls (see table 1). Administration of thyroxin increased also the rate of absorption of galactose 59 per cent and the rate of absorption of xylose 66 per cent in male and female rats respectively. Three hyperthyroid rats developed diarrhea after being fed xylose, and these were eliminated from the series.

Among the *thyroidectomized rats*, the males and females absorbed 40 and 47 per cent less dextrose, respectively, than normal rats of the corresponding sex.

Mechanism of increased sugar absorption. The following experiments were designed 1, to determine whether the influence of the thyroid hormone on absorption is due to direct action on the mucous membrane of the alimentary tract or to one of several indirect factors; 2, to ascertain the specific mechanism of increased absorption of sugars, and 3, to localize the chief site of increased absorption.

Depletion of carbohydrate. In order to test whether increased absorption of dextrose is caused by depletion of carbohydrate which takes place in the hyperthyroid state, two experiments were performed. In the first experiment, rats were deprived of food but not of water for periods of 48, 72 and 96 hours respectively before administration of dextrose. From table 2 it is learned that such fasting produced a slight decrease in the rate of absorption of dextrose, averaging 12 per cent.

In the second experiment, rats were subjected to two degrees of hepatic injury by intraperitoneal injections of a one per cent solution of phosphorus in oil, with the object of decreasing gluconeogenesis in the liver. The total doses of phosphorus, given in two equal amounts 48 and 24 hours before administration of dextrose, were respectively 6 mgm. and 8 mgm. per kilogram of body weight. Both doses of phosphorus caused definite gross and microscopic injury to the liver which has been described elsewhere (6). The smaller dose of phosphorus decreased absorption of dextrose by 16 per cent (see table 2). The larger dose caused the death of approximately half of the rats before the beginning of the absorption experiment and reduced the absorption of dextrose among survivors by 26 per cent.

Increase in basal metabolism. To determine whether an increase in the basal metabolic rate *per se* augments absorption of dextrose, the following two experiments were performed. The first experiment consisted of raising the basal metabolism of rats by intraperitoneal injections of 30 mgm. of alpha-dinitrophenol given daily for 12 days. The last injection of this drug was given 30 minutes before administration of dextrose. An increase in the basal metabolic rate of 100 per cent has been reported following administration to rats of a similar dose of alpha-dinitrophenol. This is twice the increase obtained in our animals rendered hyperthyroid

by injections of thyroxin. Such intoxication with alpha-dinitrophenol caused a lowering of the rate of absorption of dextrose by 30 per cent (see table 2).

In the second experiment the basal metabolism was increased by inducing hyperpyrexia in the rats, which were placed in a large, ventilated incubator provided with drinking water. The rectal temperatures of these rats, which before the experiment had been in the neighborhood of 38°C., were kept during the period of absorption at an average of 41.5°C. Hyperpyrexia caused a lowering of the rate of absorption of dextrose by 29 per cent (see table 2).

TABLE 2

Rate of absorption of dextrose from the digestive tract in rats under experimental conditions causing depletion of carbohydrate, increase in metabolism with a rise in the velocity of the blood flow, or hyperperistalsis

EXPERIMENTAL CONDITION	NUMBER OF RATS	RANGE OF ORIGINAL WEIGHT	LOSS OF WEIGHT AFTER FASTING	AMOUNT OF DEXTROSE FED PER 100 GRAMS OF ORIGINAL WEIGHT	AMOUNT OF DEXTROSE ABSORBED PER 100 GRAMS OF ORIGINAL WEIGHT
		grams	*per cent*	*mgm.*	*mgm.*
Normal	8	170–215	7.9	379	173 ±9*
Fasting for 48 hours	4	200–240	13.5	471	147 ±23
Fasting for 72 hours	10	186–256	16.2	411	158 ±48
Fasting for 96 hours	4	200–236	18.5	422	151 ±19
Phosphorus poisoning, 6 mgm. per kgm.	6	134–230	15.8	481	146 ±9
Severe phosphorus poisoning, 8 mgm. per kgm.	4	214–238	9.9	396	128 ±26
Dinitrophenol intoxication—30 mgm. per kgm.	4	156–162	9.4	357	121 ±25
Hyperpyrexia 40 to 43°C.	4	220–250	5.9	346	122 ±23
Administration of castor oil	4	118–140	7.8	365	147 ±16

* Standard deviation.

Increased intestinal peristalsis. As a means of learning the possible effect of increased intestinal peristalsis on the rate of absorption of dextrose (7), a group of rats were each given five drops of castor oil by mouth before the administration of dextrose. This dose had been shown to produce diarrhea as a sign of increased intestinal motility, but not until about three hours after administration. Therefore, at the end of one hour there was no diarrhea to interfere with the recovery of the residual sugar. In table 2 it is seen that feeding of castor oil resulted in a 15 per cent decrease of absorption of dextrose.

Decreased gastric emptying-time. In order to ascertain whether abnormally rapid emptying of the stomach is present in hyperthyroidism, the pylori of a number of normal and hyperthyroid rats were tied at the time the animals were sacrificed; and the residual amounts of sugar in the stomach and in the intestine were determined separately. Since the rate of gastric emptying was found to be increased by administration of thyroxin a further study was made of absorption of dextrose and of xylose in hyperthyroid rats after emptying of the stomach had been slowed by intraperitoneal injection of 0.1 mgm. of benzedrine sulphate per 100 grams of body weight 30 minutes prior to administration of sugar. When by this method the gastric emptying of hyperthyroid rats was slowed even below

TABLE 3

Influence of the gastric emptying time on the absorption of dextrose and of xylose from the digestive tract in hyperthyroid rats

EXPERIMENTAL CONDITION	NUMBER OF RATS	RANGE OF ORIGINAL WEIGHT	LOSS OF WEIGHT AFTER FASTING 24 HOURS	SUGAR USED	AMOUNT OF SUGAR FED PER 100 GRAMS OF ORIGINAL WEIGHT	GASTRIC RESIDUE OF SUGAR	INTESTINAL RESIDUE OF SUGAR	AMOUNT OF SUGAR ABSORBED PER 100 GRAMS OF ORIGINAL WEIGHT
		grams	*per cent*		*mgm.*	*per cent*	*per cent*	*mgm.*
Normal	4	190–215	7 2	Dextrose	464	72	28	168 ±12*
Hyperthyroidism	4	200–210	19.8	Dextrose	438	51	49	292 ±24
Hyperthyroidism with benzedrine	3	222–230	14.7	Dextrose	443	82	18	274 ±7
Normal	4	168–192	3.4	Xylose	270	50	50	30 ±3
Hyperthyroidism	5	130–170	19.0	Xylose	261	11	89	53 ±9
Hyperthyroidism with benzedrine	4	210–216	16.0	Xylose	227	42	58	35 ±3

* Standard deviation.

normal, the absorption of dextrose still remained high (see table 3). In contrast to this, the absorption of xylose in hyperthyroid animals under the influence of benzedrine was reduced almost to the normal rate.

The effect of phlorizin. With the object of discovering the specific mechanism by which the thyroid hormone increases the absorption of dextrose, normal and hyperthyroid rats were given a solution of this sugar to which 50 mgm. of phlorizin had been added in order to inhibit phosphorylation in the mucosa of the digestive tract (8, 9). In table 4 it is shown that administration of phlorizin drastically reduced the absorption of dextrose to approximately the same figure in both normal and hyperthyroid rats. The reduction was 79 per cent in normal and 89 per cent in hyperthyroid animals. None of these rats had sugar in either the

voided or bladder specimens of urine when they were sacrificed at the end of an hour.

Absorption of hypertonic and isotonic solutions of dextrose. In order to determine the possible participation of the stomach in the increased absorption of dextrose caused by administration of thyroxin, the absorption of an isotonic (5.4 per cent) solution of this sugar was compared in normal and hyperthyroid rats to that of the hypertonic (20 per cent) solution used in the rest of the experiments. Some of the normal and hyperthyroid animals receiving the isotonic solution of dextrose were given benzedrine so that comparison might be made of the absorption rates of an isotonic solution under conditions of slow and of rapid emptying of the stomach.

Data presented in table 5 show that administration of thyroxin increased absorption of the 5.4 per cent solution of dextrose approximately to the same degree as it did absorption of the 20 per cent solution. Gastric

TABLE 4

Influence of phlorizin on the absorption of dextrose from the digestive tract in normal and hyperthyroid rats

EXPERIMENTAL CONDITION	NUMBER OF RATS	RANGE OF ORIGINAL WEIGHT	LOSS OF WEIGHT AFTER FASTING 24 HOURS	AMOUNT OF DEXTROSE FED PER 100 GRAMS OF ORIGINAL WEIGHT	AMOUNT OF DEXTROSE ABSORBED PER 100 GRAMS OF ORIGINAL WEIGHT
		grams	*per cent*	*mgm.*	*mgm.*
Normal	8	170–215	7.9	379	173 ±9*
Normal with phlorizin	4	100–135	7.3	382	36 ±11
Hyperthyroidism	8	150–210	18.8	377	284 ±30
Hyperthyroidism with phlorizin	4	165–180	18.4	289	31 ±4

* Standard deviation.

emptying was more rapid in all rats receiving the 5.4 per cent solution of dextrose than in those receiving the 20 per cent solution. In the experiment with the 5.4 per cent solution of dextrose, 0.1 mgm. and even 0.15 mgm. of benzedrine sulphate per kilogram of weight failed to delay the emptying of the stomach in either normal or hyperthyroid rats. For this reason, the figures for absorption in table 5 are divided into two groups according to the relative rapidity of gastric emptying regardless of whether benzedrine was administered or not. From a comparison of the rates of absorption of dextrose in these two groups it is seen that, in the range of chance variation obtained in this experiment, gastric emptying-time had no influence on the absorption of an isotonic solution of dextrose.

The absorption of fats. Olive oil and oleic acid were given in doses of

approximately 1 cc. per 100 grams of body weight. Three hours later the unabsorbed residue was determined by the method of Irwin, Steenbock and Templin (10).

Figures for absorption of olive oil and oleic acid by *normal rats* are shown in table 6. From these figures it may be learned that normal animals absorbed 40 per cent less oleic acid than olive oil. Our figures on absorption of olive oil were approximately equal to those found by Steenbock et al. (11) in their experiments on rats.

In the *hyperthyroid rats*, the rate of absorption of olive oil was normal. On the other hand, these rats were found to have absorbed 115 per cent more oleic acid than the normal controls (see table 6). Consequently, the hyperthyroid animals absorbed 34 per cent more oleic acid than olive oil.

TABLE 5

Influence of the concentration of dextrose and of the gastric emptying time on absorption of dextrose from the digestive tract in normal and hyperthyroid rats

EXPERIMENTAL CONDITION	NUMBER OF RATS	RANGE OF ORIGINAL WEIGHT	LOSS OF WEIGHT AFTER FASTING 24 HOURS	CONCENTRATION OF DEXTROSE	AMOUNT OF DEXTROSE FED PER 100 GRAMS OF ORIGINAL WEIGHT	TIME ALLOWED FOR ABSORPTION	GASTRIC RESIDUE OF SUGAR	INTESTINAL RESIDUE OF SUGAR	AMOUNT OF SUGAR ABSORBED PER 100 GRAMS OF ORIGINAL WEIGHT PER HOUR
		grams	*per cent*	*per cent*	*mgm.*	*min.*	*per cent*	*per cent*	*mgm.*
Normal	4	190–215	7.2	20	464	60	72	28	168 ±12*
Normal	6	160–208	5.4	5.4	191	30	49	51	165 ±20
Normal	6	185–230	6.2	5.4	192	30	61	39	164 ±17
Hyperthyroidism	4	200–210	19.8	20	438	60	51	49	292 ±24
Hyperthyroidism	4	210–230	14.7	5.4	203	30	17	83	287 ±32
Hyperthyroidism	3	208–220	15.7	5.4	200	30	32	68	283 ±32

* Standard deviation.

The effect of phlorizin. In order to determine whether thyroxin increases the absorption of fatty acids through stimulation of phosphorylation, normal and hyperthyroid rats were given oleic acid to which 50 mgm. of phlorizin had been added in order to inhibit phosphorylation in the intestinal mucosa.

Since our hyperthyroid rats invariably developed diarrhea soon after being given a mixture of oleic acid and phlorizin, rendering inaccurate the determinations of the amount of residual oleic acid, it became necessary to counteract the intestinal hypermotility by an intraperitoneal injection of 0.25 mgm. of morphine sulphate per 100 grams of body weight given at the beginning of the experiment. Similar injections were given to normal rats receiving oleic acid and phlorizin without affecting the results

of the experiment. As an additional precaution, the dose of the irritating oleic acid was decreased by about one-fourth, which still left a large unabsorbed residue.

In table 6 it is shown that, in normal and in hyperthyroid animals, administration of phlorizin decreased the absorption of oleic acid almost to the same level. The reduction amounted to 42 per cent in normal rats and to 72 per cent in hyperthyroid rats.

The absorption of amino-acids. Alanin was chosen as a representative amino-acid because it had already been used for an absorption study in rats by Wilson (12). The dose of alanin was approximately 200 mgm. per 100 grams of body weight in the form of a 10 per cent solution. One hour after

TABLE 6

Absorption of olive oil and of oleic acid in normal and in hyperthyroid rats and the influence of phlorizin on the absorption of oleic acid

EXPERIMENTAL CONDITION	NUMBER OF RATS	RANGE OF ORIGINAL WEIGHT	LOSS OF WEIGHT AFTER FASTING	FAT ADMINISTERED	AMOUNT OF FAT FED PER 100 GRAMS OF ORIGINAL WEIGHT	AMOUNT OF FAT ABSORBED PER 100 GRAMS OF ORIGINAL WEIGHT
		grams	*per cent*		*mgm.*	*mgm.*
Normal	9	185–208	7.4	Olive oil	933	453 ±29*
Hyperthyroidism	5	200–220	14.5	Olive oil	896	444 ±32
Normal	10	175–210	5.2	Oleic acid	891	271 ±44
Hyperthyroidism	11	180–230	11.6	Oleic acid	907	593 ±125
Normal with phlorizin	8	190–206	4.6	Oleic acid	846	156 ±14
Hyperthyroidism with phlorizin	14	192–233	11.9	Oleic acid	651	168 ±33

* Standard deviation.

administration, the amount of residual nitrogen in the digestive tract was determined by the micro-Kjeldahl method.

In table 7 it may be seen that the absorption of alanin was not influenced by administration of thyroxin. The rate of absorption of alanin in our rats was very nearly the same as that reported by Wilson.

COMMENT. As shown by our data, the thyroid hormone has the property of increasing the rate of absorption of sugars from the digestive tract. Increased absorption of dextrose from the alimentary tracts of rats with experimental hyperthyroidism is apparently not caused by greater need for carbohydrate, by elevation of the basal metabolic rate, or by intestinal hyperperistalsis. A rise in the velocity of the blood-flow was also eliminated as a possible explanation for increased absorption of dextrose by

our experiments with administration of alpha-dinitrophenol and with induced hyperpyrexia.

Abnormally rapid emptying of the stomach, which was found to exist in experimental hyperthyroidism, did not account for increased absorption of dextrose but gave a satisfactory explanation for the increased absorption of xylose. The difference between the behavior of dextrose and that of xylose in this respect is explained by the fact that dextrose is absorbed with selective rapidity probably by a process of phosphorylation which increases the diffusion gradient for this sugar in the intestinal mucosa (13, 14). For this reason, dextrose in isotonic and hypertonic solutions is absorbed at a constant rate (see table 5) which is independent of its concentration in the intestine and therefore is not affected by the rate of gastric discharge. On the other hand xylose which, unlike dextrose, is not susceptible of phosphorylation and is not utilized in the body, passes through the intestinal mucosa by simple diffusion. Therefore the rate

TABLE 7

Absorption of alanin in normal and in hyperthyroid rats

EXPERIMENTAL CONDITION	NUMBER OF RATS	RANGE OF ORIGINAL WEIGHT	LOSS OF WEIGHT AFTER FASTING	AMOUNT FED PER 100 GRAMS OF ORIGINAL WEIGHT	AMOUNT ABSORBED PER 100 GRAMS OF ORIGINAL WEIGHT
		grams	*per cent*	*mgm.*	*mgm*
Normal	8	180–230	5.6	212	96 ±6*
Hyperthyroid	8	190–220	12.9	213	94 ±10

* Standard deviation.

of absorption of xylose is proportional to its concentration in the intestine, which is increased by rapid emptying of the stomach. The same presumably is true of other substances which are absorbed by simple diffusion.

Since none of the enumerated indirect actions of thyroxin, when reproduced by other means, increased the absorption of dextrose, it seemed probable that the thyroid hormone has a direct action on the mucosa of the digestive tract. Theoretically, this could be an increase in permeability of the mucous membrane or stimulation of phosphorylation. Increased permeability of the mucosa was ruled out because the rate of absorption of xylose became normal in hyperthyroid rats when gastric emptying was retarded by the use of benzedrine. If stimulation of phosphorylation is a significant factor, it would be expected that inhibiting it with phlorizin would cause a relatively greater decrease in absorption of dextrose in hyperthyroid than in normal rats. Actually the rate of absorption for dextrose in both normal and hyperthyroid animals was decreased by administration of phlorizin to that of xylose, and all differ-

ences between the two groups of rats were eliminated. This indicates that increased phosphorylation is not only an important but probably the *only* important factor in the increased absorption of dextrose in hyperthyroidism.

This theory is confirmed by the fact that the absorption of two other substances capable of phosphorylation, namely, galactose and oleic acid, was also increased under the influence of thyroxin, and that phlorizin decreased the absorption of oleic acid to the same level in normal and in hyperthyroid rats.

The unchanged absorption of olive oil in hyperthyroid rats is probably explained by the limiting factor of lipase hydrolysis which is not affected by thyroxin. This explanation is upheld by the fact that hyperthyroid rats absorbed pre-formed oleic acid faster than olive oil even though in normal rats the reverse was true.

Discovery of the mechanism by which thyroxin appears to bring about increased absorption of substances susceptible of phosphorylation raises the question as to whether this hormone increases oxidative processes in the cells in an analogous manner through acceleration of phosphorylation. Experiments are under way to test this possibility.

Ravdin (15) recently demonstrated that, whereas dextrose is not absorbed in the stomach from an isotonic solution, it is absorbed in this organ from hypertonic solutions. This gave us an opportunity to determine whether the stomach shares in the increased capacity for absorption by comparing the effect of thyroxin on the absorption of a 20 per cent solution of dextrose with that of a 5.4 per cent solution. Since the rate of absorption from both solutions was practically identical, regardless of the rapidity of gastric emptying, the conclusion seems justified that the increased absorption of dextrose in hyperthyroidism is limited to the intestine.

In contrast to the investigated sugars and oleic acid, thyroxin did not change the rate of absorption of alanin. Amino-acids are not susceptible to phosphorylation, but other than this little is known about the mechanism of their absorption.

Our work supports Verzár's theory of selective absorption through phosphorylation in the intestinal mucosa (13). The main evidence advanced by Verzár and his associates in favor of this theory is the fact that poisoning with monoiodoacetic acid or with phlorizin inhibits specifically the selective absorption of utilizable sugars and of fats, but does not interfere with the absorption of substances that are not susceptible of phosphorylation. Our findings supplement this evidence by showing that thyroxin specifically stimulated the selective absorption of utilizable sugars and of fatty acids; and that this stimulation is accomplished apparently through increased phosphorylation, because direct stimulation

of absorption by thyroxin is limited to substances capable of phosphorylation, and because this stimulation is totally inhibited by phlorizin.

Clinically, the accelerating effect of the thyroid hormone on absorption of sugars from the digestive tract probably accounts for the abnormally high dextrose tolerance curves of many patients with hyperthyroidism. The same mechanism is probably responsible for the impairment of "tolerance" to galactose observed by us in persons with this disease (1). Likewise the retarding effect on absorption of dextrose produced by removal of the thyroid gland offers a plausible explanation for the unusually low dextrose tolerance curves characteristic of patients with myxedema.

SUMMARY

1. Administration of thyroid hormone to rats markedly increased the absorption of dextrose, galactose, xylose and oleic acid in the digestive tract, but did not affect the absorption of alanin.

2. Thyroidectomy caused a considerable reduction of the absorption of dextrose in the gastro-intestinal tract.

3. Absorption of xylose in the digestive tracts of rats treated with thyroxin is increased apparently through abnormally rapid emptying of the stomach.

4. Absorption of dextrose, galactose and oleic acid in the alimentary tracts of rats treated with thyroxin is increased probably due to stimulation of phosphorylation.

5. The following conditions were ruled out as factors accelerating the absorption of dextrose in hyperthyroid rats: depletion of carbohydrate, increase in basal metabolism, a rise in the velocity of the blood flow, intestinal hyperperistalsis, shortened emptying time of the stomach, and increased permeability of the mucosa.

6. Increased absorption of dextrose in the digestive tract appears to take place chiefly in the intestine.

7. These findings indicate that the thyroid hormone influences absorption mainly by increasing phosphorylation, and by stimulating gastric emptying.

8. The discovery that thyroxin specifically stimulates the absorption of substances susceptible of phosphorylation and that phlorizin inhibits this stimulating action, confirms Verzár's theory of selective intestinal absorption through phosphorylation.

9. Clinically, altered absorption is an important factor in producing abnormally high sugar tolerance curves in patients with hyperthyroidism, and unusually low sugar tolerance curves in patients with myxedema.

NOTE. The authors wish to acknowledge their debt to Dr. R. I. Pencharz for performing the thyroidectomies, and to Dr. E. A. Anderson for determinations of the basal metabolism.

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THE EFFECT OF PREGNANCY AND LACTATION ON GROWTH IN THE RAT

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Received for publication April 20, 1938

Actual controlled data regarding the effect of pregnancy on growth are limited in the literature. Pregnancy too early in the life of all mammalian species has been generally considered harmful to the mother. Slonaker (1927) inferred that early pregnancy hindered growth in the rat though he showed that breeding females exceeded virgins in body length. His data on body weight are difficult to evaluate as the averages include pregnant and nonpregnant animals. There is however some evidence that early pregnancy is not harmful. Eckles and Swett (1918) with dairy heifers and McKenzie (1928) with sows showed, for example, that early pregnancy had no demonstrable effect upon growth.

Cole (1937) obtained very early pregnancy in young rats before normal sexual maturity by stimulating precocious sexual development through the injection of mare gonadotropic hormone. The interesting observation was made that this excessive burden on these very young rats was carried with an increase in growth as determined by body weight and Hain (1932) has similar findings for single pregnancies in older rats.

From these observations and from others made in husbandry operations—particularly the increased gains of pregnant heifers in the feed lot—it was decided to test the effect on growth of repeated pregnancy and of repeated pregnancy with lactation in young rats with littermate controls kept in the nonpregnant condition but with identical environment.

EXPERIMENTAL. The following groups of rats were used in these experiments:

Group 1, consisting of 10 non-bred controls.

Group 2, consisting of 10 littermate sisters of groups 1 and 3. They were maintained through 2 to 9 gestation periods without lactation, the young were all destroyed the day of littering.

Group 3, consisting of 10 littermate sisters of groups 1 and 2, maintained through 5 to 8 gestation and lactation periods. At birth the litters were reduced to 4–6 and nursed for 21 days.

Group 4, consisting of 5 rats carried through 9 to 10 gestation and lactation periods.

Group 5, consisting of 5 littermate sisters of group 4. They were maintained through 3 gestation and lactation periods after which they were not again bred but served as controls to the group 4 animals.

Group 6, consisting of 10 non-bred rats serving as controls to group 7 and so maintained that daily weighing of food consumption and daily weight could be recorded.

Group 7, consisting of 10 littermate sisters of group 6, so maintained through 1 or 2 gestation and lactation periods that daily weighing of food consumption and body weight could be recorded.

Four of the animals in group 1 were injected with 8 rat units of mare gonadotropic hormone but not bred, and the remaining 6 were not injected. This variation had no noticeable effect on the growth curve of the animals in this control group.

In order for the effect of pregnancy to be obtained early in the growth cycle, all the bred rats except those in group 7 were injected with 8 rat units of mare gonadotropic hormone at 28 to 31 days of age. Four of the animals in group 2, two of those in group 3, and all those in groups 4 and 5 were successfully bred and impregnated within 72 hours after the injection. This variation is not uncommon in rats of this age when injected with mare gonadotropic hormone. The remaining animals in groups 2 and 3 were bred at the first normal oestrus, which occurred between the 48th and 88th days of age.

The group-7 rats were allowed to attain normal sexual maturity. They were bred between the 50th and 80th days of age.

In all cases the breeding animals were rebred as soon as possible after parturition or the end of lactation without any definite period of time being allowed to elapse. For example, rats of group 2 the day after littering and those of group 3 the day after weaning were smeared daily and bred at the first oestrus. If pregnancy did not intervene, the procedure was continued until pregnancy was established.

All the rats were fed *ad libitum* McCollum diet I and also greens in the form of fresh alfalfa twice weekly. They were for the most part kept in individual cages.

All the animals were weighed with the controls on the day bred, within 24 hours after littering, and on the day of weaning except groups 6 and 7 which were weighed daily.

All the rats in the first five groups were killed at the conclusion of the experiment, and nose-tail and nose-anus lengths measured. At autopsy the adrenals, thyroids, and pituitary glands were removed, weighed and saved for histological study. At parturition in several cases, the fully developed mammary glands of rats in the bred groups were dissected out and weighed. The breeding animals were killed on the day of parturition except when they could not again become pregnant. This only occurred

in group 2 from genital infection, the cause of which is not clear but may have resulted from daily vaginal smearing beginning the day after parturition when the cervical canal is open. The controls were killed with the second animal of the trios in groups 1, 2, and 3.

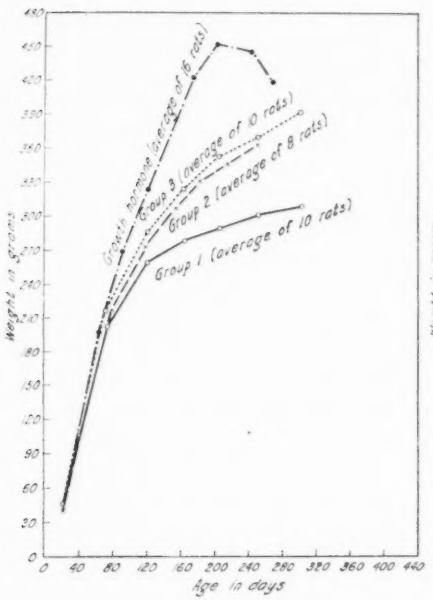
Group 3 (average of 6 rats)
Group 3 (average of 10 rats)
Group 2 (average of 8 rats)
Group 1 (average of 10 rats)
Weight in grams
Age in days

Fig. 1

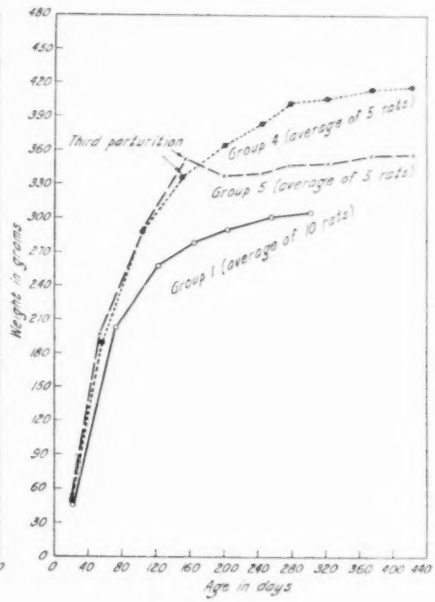

Fig. 2

Fig. 1. Growth curves of rats in groups 1, 2 and 3. The points on the curves for groups 2 and 3 represent the weight of the rats on the 21st day of age and after each parturition; those for group 1 represent weights made at the same ages as their littermate sisters in group 3. The growth curve of rats receiving growth hormone between the 60th and 223rd to the 263rd days of age is also given for comparison.

Fig. 2. Growth curve of rats having nine pregnancies (group 4) as compared with that of rats not bred after the third parturition (group 5) and to non-bred controls (group 1). The weights were taken on the 21st day of age and within 24 hours after each parturition for group 4 whereas rats in group 5 after their 3rd parturition were weighed at the same time as their littermates in group 4.

Results. The growth curves of groups 1, 2, and 3 are shown in figure 1. With the establishment of the first pregnancy, growth is accelerated; and the difference in weight of the breeding animal over the virgin control is widened with each succeeding gestation.

This increase, at the end of the first pregnancy in groups 2 and 3, is not statistically significant for this relatively small group. Consequently

another lot of 52 animals were injected at 28 to 30 days of age; of these 26 were bred, and the remaining 26 littermate sisters were kept as controls. Sixteen of the pregnant rats with their controls were killed between the 18th and 21st days of pregnancy, the uterus and its contents being removed in each and the remainder of the carcass weighed. The other animals in this lot with their controls were weighed immediately after parturition. There was an average weight difference of 10 grams in favor of those that had been pregnant. By Fisher's paired method for the standard error of the mean this weight difference was found to be significant ($0.01 < P < 0.02$).

TABLE 1

Weaning weights, number of pregnancies and autopsy data for groups 1, 2 and 3

LITTER NUMBER	WEANING WEIGHT			NUMBER OF PREGNANCIES		AGE AT AUTOPSY			WEIGHT AT AUTOPSY			NOSE-ANUS LENGTH AT AUTOPSY		
	Group 1	Group 2	Group 3	Group 2	Group 3	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
	gms.	*gms.*	*gms.*			*days*	*days*	*days*	*gms.*	*gms.*	*gms.*	*cm.*	*cm.*	*cm.*
1	46	47	50	6	8	383	383	357	298	332	341	22.1	22.1	22.1
2	54	51	53	2	7	387	364	387	338	457	486	23.5	24.4	25.5
3*	40	45	36	9	7	357	350	357	325	402	372	22.4	23.3	23.2
4	47	46	47	6	8	382	382	358	325	321	370	22.4	22.4	23.2
5	48	44	50	9	6	345	345	342	360	462	420	23.3	24.0	23.3
6	52	42	46	6	6	330	330	321	337	378	398	22.3	23.2	24.0
7	41	40	44	9	7	301	301	306	308	368	493	22.2	23.3	24.1
8	44	41	38	9	5	338	338	327	290	389	363	22.2	23.6	23.5
9	47	35	46	5	7	352	352	336	276	376	387	22.5	24.1	23.3
10	40	39	40	4	6	329	329	313	252	302	338	21.3	22.5	22.4
Ave.	45.9	43.0	45.0			350	347	340	311	379	397	22.42	23.29	23.46

* There was no group 2 rat for this litter while there were two for litter 4. Thus the figures for the extra animal in litter 4 are included in this column.

Little variation is observed between the lactating and non lactating group. The effect of lactation will be taken up later when the daily weighed groups are discussed.

Table 1 shows the weaning and final individual weights, the number of pregnancies and the age of the animals. It also gives the nose-anus lengths of the animals at autopsy in groups 1, 2, and 3. In every case the bred animals exceed their littermate controls in weight except in litter 4, in which the group 2 rat was 4 grams lighter than the group 1 control. At the end of the 6th pregnancy (fig. 1) there was an average difference of 81 grams between group 3 and the controls. The difference between group 2 and the same controls was 59 grams. The smaller difference is

due to the fact that this nonlactating group completed their 6th pregnancy on the average 49 days younger than their littermate group 3 sisters. At autopsy, when the number of pregnancies varied from 2 to 9, the average weights of these three groups were 311, 379, and 397 grams respectively. Each of the first six pregnancies contributed fairly uniformly to the excess gains made by the pregnant rats over their controls, the one exception being the relatively small excess gains made between the 4th and 5th parturitions (table 2).

The animal (G 3668, litter 2) having only 2 pregnancies weighed 457 grams at autopsy as compared with its control weighing 338 grams. This increased weight was due, we believe, to the regularity of her breeding followed by typical pseudopregnancy periods with cessation of the cycle until almost the time of the placental sign on the 13th day after mating,

TABLE 2

Gain of group 3 rats through successive pregnancies and lactations as compared to littermate non-bred group 1 rats over the same time interval (ten pregnant and ten control rats)

PERIOD	GAIN OF PREGNANT RATS	GAIN OF CONTROL RATS OVER SAME TIME INTERVAL	EXCESS GAIN OF PREGNANT RATS
	grams	*grams*	*grams*
Between weaning and 1st parturition, 21st to 73rd days.	172	157	15
Between 1st and 2nd parturition, 73rd to 122nd	69	56	13
Between 2nd and 3rd parturition, 122nd to 166th	38	20	18
Between 3rd and 4th parturition, 166th to 208th	29	9	20
Between 4th and 5th parturition, 208th to 253rd	16	13	3
Between 5th and 6th parturition, 253rd to 303rd	22	9	13
Total excess gain			82

when oestrus would again appear. This occurred thirteen times over a 9 month period. This rat showed rapid weight increases after each breeding followed by a period of growth stasis which is in accord with Slonaker's findings (1929) during pseudo-pregnancy.

Evidence that pregnancy induced increased skeletal growth is found in the nose-tail and nose-anus measurements, in which the pregnant animals in groups 2 and 3 averaged 0.87 and 1.04 cm. in nose-anus and 1.42 and 1.55 cm. in nose-tail measurements respectively over the controls.

Figure 1 also presents data obtained with a group of 16 non-littermate rats receiving growth hormone (Kleiber and Cole, unpublished). Evidently pregnancy is intermediate in its growth stimulus between that occurring in virgin controls and rats regularly treated with maximal stimulating amounts of growth hormone. The fall at the end of the curve in

the latter is due to the stopping of the hormone injections. Evans and Simpson (1931) show that females are more responsive to growth hormone than males. This is only true, however, provided the control females are kept in the virgin condition.

Figure 2 shows the growth curves of rats in groups 4, 5 and 1. The latter are presented again in order to compare the growth of nonpregnant controls. The animals in group 4 were carried through 9 gestation periods. Those in group 5 were carried through 3 pregnancies simultaneously with the first 3 pregnancies of group 4, and thereafter the breeding of the group 5 animals ceased. This was done to ascertain if growth would simultaneously cease with the cessation of gestation periods. An average of only 5 grams' increase was obtained over the following 270 day period. The group 4 animals gained an average of 80 grams during the period. The nose-anus measurement of group 4 exceeded that of group 5 by 0.5 cm., the nose-tail by 1 cm.; the 3 pregnancies of group 5 narrowed these average differences.

The average weight of the mammary glands of five rats in these experiments on the day of littering was found to be 22.3 grams. The variation in weight was quite wide, ranging from 14.7 to 37 grams. This is accounted for by the amount of suckling that had taken place previous to autopsy. No suckling had occurred in the one weighing 37 grams.

These mammary gland weight data show that the group 5 rats which made an average gain of 5 grams after the third parturition actually gained this amount plus the weight of the mammary gland which atrophied to a negligible weight. The group 4 rats, weighed each time after parturition, when the mammary gland was fully developed, credit the entire gain of 80 grams to body tissues other than mammary gland. Figure 2 shows that rate of gain slowed materially after about the 280th day.

Food consumption during pregnancy and lactation. As stated above, the food consumption data were obtained on groups 6 and 7 which consisted of animals bred at the time of sexual maturity or shortly thereafter. The rats were placed in individual cages and were fed in cups with a sloping rim to lessen spilling. As the feed was weighed daily and as the bottom of the cage was regularly examined for spilled feed, the data obtained are fairly reliable. On days when appreciable spilling had obviously occurred, the weighings for the particular rat were not included. There was a greater tendency for spilling during lactation than at any other time, and it may be that our figures for this period are somewhat high.

The increased growth during pregnancy is of course accompanied by an increased food intake (fig. 3). The most striking observation is the short interval of time between the establishment of pregnancy and increased food consumption. On the second day after breeding, when the demand

of the embryos is negligible, a distinct rise occurred, which remained fairly constant until about the middle of pregnancy. This was so conspicuous in most cases that the existence of pregnancy or pseudopregnancy could be recognized by it. Later in pregnancy a further increase took place. The latter is more definitely due to the rapidly increasing size of the fetus. The former is probably due to hormonal factors, whose liberation may be initiated by the act of mating. In the rat we know that pseudopregnancy can follow sterile copulation. Barelare and Richter (1938) showed a definite and very constant increase in sodium chloride intake in pregnant rats given a free-choice method of selecting solid foods and solutions.

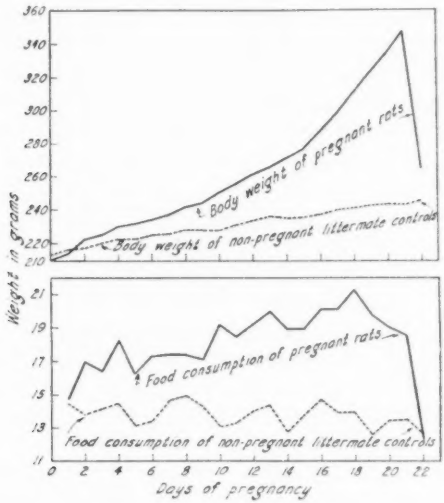

Fig. 3. Average daily food consumption and gain in body weight of 14 pregnant rats (group 6) as compared with those of 14 nonpregnant littermate controls (group 7).

This increase amounted to twice as much during the first 10 days of gestation as during the 10 day period immediately preceding fertilization. We doubt that the increased food consumption in our animals can be ascribed to the increased desire for salt present in McCollum diet I.

On the day of parturition the food consumption falls below that of the controls. There is a steady rise from this point during the lactation period, however, and by the 12th day after parturition the lactating rats eat about three times as much as nonlactating controls. The food consumption remains fairly constant from this point to the end of lactation on the 21st day after parturition. During the latter half of lactation the

daily food consumption is apparently about twice that found in the latter part of pregnancy. On the 12th day of lactation for example the average daily food consumption was 41.4 grams as compared with 21.3 grams on the 18th day of pregnancy.

During early lactation the gains in weight made by the lactating rats exceed those of their nonlactating controls, but during the latter part the increased milk production prevents further gains. Consequently, the lactating and nonlactating rats made equal gains over the entire period. These data, having been obtained on young growing rats, may not be applicable to older animals.

Discussion. Although the genetic constitution of an animal does predominate in determining ultimate body size, the data herein presented show that pregnancy is likewise an important factor provided the animal has access to sufficient amounts of an adequate diet. There are other physiological means of stimulating growth, the most striking of which is the injection of growth-hormone preparations from the anterior pituitary (Evans and Long, 1921). Ovariectomy before maturity also results in increased body weight in some species. Freudenberger and Hashimoto (1937) have reviewed the literature for the rat. Although ovariectomized rats at 70 days are also longer than intact control animals (Freudenberger and Hashimoto, 1937), this is not true at six months (Freudenberger and Billeter, 1935). Finally Donaldson (1932) has submitted data indicating that exercise in the rat produces increased body size.

The injection of growth hormone brings about an increased appetite, and this is also true of pregnancy and lactation. Possibly the copulatory act at the inception of pregnancy brings about an increased pituitary function through the intermediary of the nervous system. The fact that appetite increases so promptly after copulation lends support to this view, although a hormonal action on the pituitary cannot be completely ruled out. During lactation the pituitary, no doubt, is likewise involved in the increased appetite. Is there a greater secretion of growth hormone during lactation? If the lactogenic hormone were responsible for the appetite, it would seem that the lactogenic hormone would also be a growth hormone for an important essential of increased growth is an increased appetite.

CONCLUSIONS

The data demonstrate that pregnancy stimulates skeletal and tissue growth in the rat beyond that found in non-bred littermate controls. This fact is evidenced even during the first pregnancy in precociously matured females. The excess gains made by the pregnant rats remain fairly constant for the first six pregnancies, after which further pregnancies have less effect. The excess gains are made, for the most part,

during pregnancy, although rats suckling 4 to 6 young continue to gain as rapidly as non-bred controls.

The excess growth during pregnancy is accompanied by, and presumably dependent upon, an increased food consumption manifested by the second day after conception. It is postulated that the copulatory act brings about a nervous stimulation of the anterior pituitary, resulting in the secretion of one or more hormones involved in inducing an increased appetite.

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REMOVAL OF FOREIGN SUBSTANCES BY THE LYMPHATICS OF THE SNAKE LUNG

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Received for publication April 26, 1938

The rôle of the lymphatic system in removing proteinized fluids and foreign substances from the lungs is not known as it has so far been impossible to collect lung lymph in mammals. Since this can be done in the snake, we have made use of this lower form to secure data on the problem, which may be of value in the interpretation of mammalian experiments.

In the Indigo snake only the left lung is present. It is a simple tubular structure, the dorsal part of which consists of a sheet of alveolar tissue folded over at the right edge in which are located the vena cava and the pulmonary artery and vein. The remainder of the tube is a thin serous membrane lined by ciliated columnar epithelium. The lymphatics are perivascular (Chapman and Conklin, 1935). These relationships are shown in cross section in figure 1. Branches of the pulmonary artery and vein that pass over the lung tissue have, continuing with them, a lymphatic sheath that can be traced to the fairly small blood vessels. The two lymphatics about the three large blood vessels open into the jugular lymph sac that lies at the base of the heart.

PHYSIOLOGY OF LUNG LYMPHATICS: MATERIAL AND METHODS. The 48 Indigo snakes used for this study belong to the species Drymarchon corais melanarus of the family Colubridae. The animals, averaging about 800 grams in weight, were anesthetized with 1 cc. of 25 per cent urethane per 85 grams of body weight by stomach tube.

An incision to the right of the midline exposed the blood vessels. The lymphatic sheath was punctured with a hypodermic needle and the lymph collected in a capillary tube or pipette. All manipulations were done under a binocular microscope. When 20 to 30 cc. of 0.8 per cent saline were given by stomach tube the day before the experiment, abundant collections of lymph could be quickly and easily made, provided respiration was not depressed by an excess of anesthetic.

EXPERIMENTAL RESULTS. *1. Hemoglobin.* Six experiments were performed using as the injection fluid a hemoglobin solution prepared from snake blood by diluting one volume of blood with four of water (20 per cent solution) and either centrifuging it or allowing it to stand over night

in the ice box. In some later work, the actual hemoglobin content of snake blood was found to be 8.88 grams per 100 cc. of whole blood, average of five determinations by the improved Newcomer method. The hemoglobin in the test solution was therefore approximately 1.78 per cent.

A series of standard dilutions was made up in order to determine the concentration of laked blood in the collected lymph samples. The 20 per cent solution of whole blood was diluted with snake plasma to make the following percentages: 20, 10, 5, 4, 3, 2.5, 2.0, 1.5, 1.25, 1.0, 0.625, 0.5, and pure plasma. These were set up in a series of capillary tubes of the same inside diameter, and the tubes sealed at both ends.

After a sample of normal lymph had been procured, 4 cc. of the solution containing the hemoglobin were injected into the lung by inserting a small catheter down the trachea. The collections of lymph from the pulmonary-vein lymphatic were then made at five minute intervals and continued for

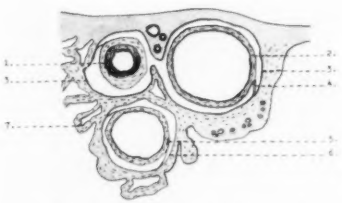

Fig. 1. A cross section of the three large blood vessels at the edge of the snake lung, showing the arrangement of the lymphatics about them. The lymphatics have a double endothelial wall, one applied closely to the blood vessel, the other lining the outer wall.

Key: *1*, pulmonary artery; *2*, vena cava; *3*, common lymphatic about the vena cava and pulmonary artery; *4*, trabecula between the outer and inner lymphatic walls; *5*, pulmonary vein; *6*, lymphatic about the pulmonary vein; *7*, lung tissue.

more than two hours in some cases. Each capillary tube was sealed and compared with the standard series to determine the percentage concentration of laked blood in the lymph.

After the injection the respiration became deeper and sometimes was almost convulsive; it gradually subsided and toward the end of the experiment became irregular. The increased breathing helped diffuse the solution throughout the lung tissue, and in every case the maximum concentration of laked blood found in the lymph was proportional to the vigor of the respiration.

Table 1 gives the results of the six experiments. It shows clearly that the time of first appearance of the hemoglobin varies somewhat in the different animals, and that a maximum concentration is reached followed by a fall. The figures in the last column are the average results for the six determinations. They show a maximum concentration of 1.25 per

cent laked blood after 45 minutes, followed by a slight decrease to a level which is maintained for 40 minutes. From this time on there is a continued gradual decrease. It was noted that as the concentration of hemoglobin in the lymph fell, the lung began to "clear", and that often by the next day the color was nearly normal, i.e., a light pink.

TABLE 1

Concentration of laked blood in lymph from the pulmonary-vein lymphatic

The last column is an average of the series of six experiments

TIME AFTER INJECTION	PER CENT CONCENTRATION OF LAKED BLOOD IN LYMPH						
	Experiment 19*	Experiment 20	Experiment 21	Experiment 22	Experiment 23	Experiment 24	Average
minutes							
5						0.0+	
10	0.0						0.0
15	0.0+	0.0	0.0				
20	0.625	1.25	0.625		0.0		0.43
25	1.0				0.625	0.0+	
30	1.0+	1.25			0.5	1.0	0.78
35	*1.25*	3.0				1.25	
40	1.0+					1.25	0.98
45	1.0	*4.0*	1.0			*1.25*	*1.23*
50	1.0		1.0+	0.0	0.0	1.25	0.96
55			*1.25*	0.625		1.25	1.05
60	1.0	1.25	1.0	1.0	0.0+	1.0	0.89
65				1.5			
70	1.0			1.5	0.625	1.0	0.88
75		0.0	0.625	2.0			
80	1.0					1.0	0.93
85			0.5	*2.5*	*1.0*		0.97
90	0.625					1.0	0.88
95			0.5	2.0			
100					0.625	1.0	0.64
105							
110		0.0	0.0+	1.25		0.625	0.37
115					0.0		
120						0.625	0.25
125				0.625			
130							0.1
135						0.625	

* Time is one minute less than indicated.

2. Vital red. In the second series, five experiments were performed using as the injection fluid a 1 per cent solution of Vital Red, Ehrlich's H.R. A set of standard dilutions was made up as in the preceding series including the following percentage concentrations: 1.0, 0.5, 0.4, 0.3, 0.25, 0.2, 0.15, 0.1, 0.05, 0.025, 0.01, 0.005, and pure plasma.

TABLE 2

Concentration of Vital Red in the lymph from the pulmonary-vein lymphatic
The last column is an average of the series

TIME AFTER INJECTION	PER CENT CONCENTRATION OF VITAL RED IN LYMPH					
	Experiment 26	Experiment 27	Experiment 29	Experiment 30	Experiment 33	Average
minutes						
5				0.005		
10				0.01		0.002
15		0.0				
20		0.0+		0.01		0.0022
25	0.0	0.005				
30	0.0+	0.025		0.01		0.007
35		*0.01*		*0.05*		
40	0.0+	0.005		0.025		0.006
45		0.0025			0.0	
50		0.0+	0.0	0.01		0.0034
55			0.001		0.0075	
60		0.0+				0.0045
65			0.001		0.0075	
70		0.0+		0.01		0.0051
75	0.005		0.001		*0.01*	
80	0.005			0.005		0.0038
85	0.007		0.001		0.005	
90	0.008			0.01		0.0048
95	0.01		0.001		0.005	
100				0.01		0.0063
105	0.02		0.002		0.005	
110				0.01		0.0074
115			0.002		0.001	
120	*0.025*		0.0025	0.01		0.0077
125					0.001	*0.0077*
130			*0.0025*	0.01		0.0077
135					0.005	
140			0.0025			0.0055
145					0.005	
150						0.0049
155					0.005	
160					0.005	0.0042
165			0.0			
170						0.0025
175						
180	0.01					

From table 2 it may be seen that the time of the first appearance of the Vital Red is even more variable than was the hemoglobin, and that it takes longer for the dye to disappear from the lymph after the first maximum peak is reached. The average results (last column) show two maximum

peaks, the first reached after 30 minutes with a concentration of 0.007 per cent; the second, after 120 minutes with 0.0077 per cent. Between these two maxima, a somewhat irregular plateau level of concentration is maintained, and after the second maximum, there is a gradual but decided fall in concentration.

In this series the respiration never became "convulsive" as it had in the hemoglobin experiments, but remained much slower and more even. The tracheal membrane was cut shortly after injection of the Vital Red into the lung and a large glass tube inserted, because great plugs of mucus containing the dye were constantly being moved up the trachea by ciliary action. The glass tube prevented blockage of the air passage and the even character of respiration was insured.

Again it was noted that the lung became a very dark red as the injected dye diffused throughout the organ. However, as soon as the concentration of Vital Red in the lymph increased, the color of the lung subsided, and the congestion was noticeably relieved.

TABLE 3

Time of passage of carmine from the lung into the pulmonary-vein lymphatic

	EXPERIMENT 34	EXPERIMENT 35	EXPERIMENT 36	EXPERIMENT 37	AVERAGE
Time when carmine was first noted	Less than 1 minute	10 minutes	Less than 1 minute	8 minutes	4.5 minutes

3. Carmine. After ascertaining that substances in solution were cleared from the lung in a few hours, it was decided to find out if particulate matter was also disposed of as readily and to find the time when these particles first appeared in the lymph from the pulmonary-vein lymphatic.

Four cubic centimeters of a 2 per cent carmine suspension in 0.8 per cent saline were injected into the lung. The lymph was collected from the lymphatic in a capillary pipette and the fluid blown out on a glass slide. Collections were made immediately after injection, after two, three, and five minutes, and from then on usually at five-minute intervals. The slides were examined at leisure for the tiny red carmine granules which could easily be seen with a high power objective.

Table 3 gives the time of first appearance of the carmine in four experiments.

Although the variation is fairly wide, the average time of 4.5 minutes is consistent with the results obtained below. Again respiration was a factor that greatly influenced the entrance of these particles into the lymph vessels; if the breathing was slow and shallow, the time was increased.

4. Calcite. A 2 per cent suspension of calcite (powdered $CaCO_3$) was used as the injection material for this set of four experiments. In the identification of the particles a dark field microscope was used; the particles are luminous and can be seen as brilliant crystals in the dark field (Field and Drinker, 1936).

The time when the calcite first appeared is recorded in table 4. There was a variation of five minutes in the four determinations, but the average time of 4.25 minutes checks closely with the average for the carmine. The respiration was nearly normal in all the experiments.

5. Hydrokollag. A graphite preparation, Hydrokollag 300, was prepared by the method of Drinker and Churchill (1927) as modified by Higgins and Murphy (1928). For injection into the lung, this suspension was diluted 1:1 with 0.8 per cent saline. This heavy black mass usually

TABLE 4

Time of passage of calcite from the lung into the pulmonary-vein lymphatic

	EXPERIMENT 38	EXPERIMENT 43	EXPERIMENT 44	EXPERIMENT 45	AVERAGE
Time when calcite was first noted	7 minutes	3 minutes	2 minutes	5 minutes	4.25 minutes

TABLE 5

Time of passage of Hydrokollag from the lung into the pulmonary-vein lymphatic

	EXPERIMENT 39	EXPERIMENT 40	EXPERIMENT 41	EXPERIMENT 42	AVERAGE
Time when Hydrokollag was first noted.....	3 minutes	5 minutes	5 minutes	3 minutes	4.00 minutes

slightly increased the breathing. The results of these determinations show the least wide variation, two minutes, with an average for the series of four of 4.00 minutes.

In comparing the average results (last columns of tables 1 and 2) for the hemoglobin and Vital Red, it is noted that there is a rapid increase in the concentration after the injection of the fluids into the lung, the peaks appearing between 30 and 45 minutes later. Thereafter a slightly lower level is maintained for a time, followed by a second peak and a more pronounced fall in concentration. For some reason, possibly because it does not increase respiration to any great extent, the Vital Red is not removed as soon as the hemoglobin and therefore is found in the lymph for a longer time.

The last three series of experiments determine the time in which sub-

stances may first be expected to appear in the lymphatic vessels after the deposition of this material in the lung. The three averages vary only one-half minute, fixing the time at 4.25 minutes after injection.

Discussion. Since the substances that form true solutions very easily found their way through the lymphatic wall, even though the molecular weight be as great as that of some of the proteins, e.g., hemoglobin (table 6), it would seem that the limit for size of particles which can get into the lymphatics is well up in the microscopic range. Besides those substances in table 6 already discussed are included starch grains. In a preliminary experiment starch grains (potato starch) that had been made blue by treating them with iodine in potassium iodide solution were injected into the snake lung in a suspension of 0.8 per cent NaCl. None of these grains which measure 5μ and slightly less could be found in the lymph collected from the pulmonary-vein lymphatic, either free or phagocytized by any leukocytes present. Since calcite and carmine granules were found in

TABLE 6

Molecular weights and measured sizes of substances injected into the lung of the snake

	MOLECULAR WEIGHT	MEASURED SIZE OF VISIBLE PARTICLES
Hemoglobin (human)	67,000	
Vital Red (Trypan Red)	1,003	
Carmine		0.5–1.5μ
Hydrokollag		0.5μ and less
Calcite		1–2μ
Starch grains		5μ and less

abundance in the lymph and starch grains were not, the upper size limit for penetration of snake lymphatics must be placed at about 2μ.

Field and Drinker (1936) found that such inert particles as graphite, measuring 1μ, and calcite, 1 to 2μ, were found in the lymphatics of dogs very soon after injection into the blood stream. In the case of motile organisms, the size was somewhat greater; microfilariae measuring 40μ in length and 5μ in width were seen in the lymph of the thoracic duct in great numbers after an intravenous injection.

In still more recent work, Drinker, Warren, and MacLanahan (1937) have found, contrary to their expectations, that in the lungs of dogs, horse serum, crystallized hemoglobin, and crystallized egg albumin are not removed by the lymphatics but are transferred across the pulmonary epithelium from the alveoli directly into blood vessels. However, inorganic particles and bacteria somehow do make their way through lung tissue into the lymphatics in the breathing lung.

In the snake the lymphatic system is by no means the only way in which

foreign substances are removed from the lung. Very early in the work it was noted that streams of the injected material were moved rapidly up the under surface of the membrane that lies over the ventral part of the lung cavity. Histological sections proved that this was due to the cilia which line the membrane. These streams were moved up through the trachea, the material being plentifully mixed with mucus on the way. When it reached the mouth, the mixture was swallowed.

By splitting the membrane over the lung, the alveolar tissue could be observed, and also the method by which a large portion of the injected substance was removed. The cilia along the ridges which form a mesh arrangement sent the particles or the dye to the two large ridges that run cranio-caudally on either side of the lung. The material was then moved along these ridges or up the membrane and finally through the trachea to the mouth. When all the injection mass that was actually in contact with these ciliated areas had been removed and only some was left deep down in the alveolar sacs between the ridges, the lung could be seen to contract in isolated areas, forcing the foreign material out to the ciliated ridges which were drawn close together. The contraction of these areas was slow and lasted as long as 30 minutes, characteristic of the smooth muscle in the ridges.

In describing the ciliary action in the lungs of mammals, Barclay, Franklin and MacBeth (1937) state that they could watch the movement of particles up the bronchi into the trachea. Using decerebrate cats, Barclay and Franklin (1937) were able to follow by means of radiographs the course of India ink injected into the right lobe of the lung through the thoracic wall. Although it was unlikely that the injected material went into bronchioles directly, a large portion of the ink appeared at the distal end of the tracheal cannula within 14 minutes after traveling a total distance of more than 9 cm.

That part of the injected mass not got rid of by mechanical means may be removed from the lung of the snake by either one of two methods. A very small fraction of it is undoubtedly engulfed by the phagocytic cells of the lung, and histological sections showed some of these cells actually in the process of taking up particulate matter. These cells were comparatively few in number and could not dispose of any great quantity of foreign material. Moreover, the particulate material could be identified in the lymph of the pulmonary-vein lymphatic in 4.25 minutes after injection into the lung. It is unlikely that phagocytic cells could move the particles at such a rate when blood smears made an hour or more after the lung had been injected showed these same particles free some time before they could be found in any leukocytes.

The greater part of the material remaining in the lung is removed by the lymphatics after it has passed through the lung tissue to the capillaries.

In the histological preparations black particles of graphite could be seen within the tissue but not in definite relation to the cells themselves, and in the lymph spaces about the larger venules the black particles could often be made out.

In conclusion, it may be pointed out that respiration is a factor that plays an important rôle in moving substances through the lung of the snake. In the cases where respiration failed, not only was very little or none of the injected substance found in the lymph, but the movement of the lymph itself was interfered with. The rate at which these substances entered the lymphatics was also affected by the breathing.

SUMMARY

1. The structure of the snake lung is described. In the Indigo snakes used there is only a left lung present, which consists of a sheet of alveolar tissue covered on the ventral surface by a membrane. The ridges, between which lie the alveolar sacs, are ciliated, as is the membrane over the rest of the lung. In the ridges are bands of smooth muscle.

2. Substances such as hemoglobin and Vital Red that form a true solution in water, are readily removed from the lung by the lymphatics, the maximum concentration of these two being reached at 45 and 30 minutes, respectively, after injection into the lung.

3. Particulate matter is also removed by the lymphatics of the lung and may be first observed in the lymph about 4.25 minutes after injection. The largest particles that can get through the walls of the lymphatics are about 2μ in size.

4. A great deal of foreign substance is removed from the lung by mechanical means. The lung tissue contracts in areas, forcing the material from the alveolar sacs out to the ridges, and from there it is carried up through the trachea by ciliary action to the mouth.

5. The part of the injected mass that is removed from the lung by the lymphatics gets through the lung tissue in one of two ways. Some of it is picked up by the phagocytic cells of the lung. The greater portion passes through the lung tissue to the lymphatics. This last method is the more important one because four and a quarter minutes is too short a time for the phagocytic cells to have deposited the particles in the lymphatic vessels.

6. Respiration is an important factor in moving matter through the lung of the snake and getting it into the lymphatics; in almost every instance the concentration and the speed of entrance was proportional to the rate and depth of respiration.

The authors wish to express their appreciation to Dr. C. K. Drinker of the Harvard School of Public Health, Boston, for his interest and sugges-

tion of this problem, and for the preparation of calcite used in one series of experiments.

We also wish to thank Miss Lois Levy for the histological preparations of the lung.

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THE EFFECTS IN MAN AND DOGS OF MASSIVE DOSES OF INSULIN ON THE COMPOSITION OF THE BLOOD SERUM

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Received for publication May 2, 1938

The so-called "insulin shock therapy" for schizophrenia has come into extensive use during the past year but, so far, little has been reported on chemical alterations in the blood resulting from this drastic treatment. This paper is concerned with the concentrations of sodium, potassium, non-protein nitrogen and proteins in the blood serum.

Subjects. The principal experimental series consisted of studies on 6 physically normal males with schizophrenia. These men had been in the Rochester State Hospital for many months and were maintained on the regular institution diet which was rather low in nitrogen. They were accustomed to the experimental procedures. All studies were performed in the morning in the fasting state and in bed rest.

Comparative studies were carried out on dogs which had been in the laboratory for several months and were accustomed to the procedures of receiving injections and blood sampling.

METHODS. Blood was drawn from an arm vein immediately before insulin injections and again at the height of the insulin reaction. In five cases a third sample was taken after the reaction showed signs of abating. In one case blood samples were taken during convulsions and immediately after convulsions had been stopped by the intravenous injection of 20 cc. of 50 per cent glucose solution.

In all cases blood was drawn into an oiled syringe and no stasis whatever was used. The blood was immediately transferred to large centrifuge tubes, allowed to clot for 1 hour and then centrifuged. The serum was separated off within 2 hours in all cases.

Insulin was administered in single injections of 20 to 130 units.

Non-protein nitrogen and total nitrogen were both determined by a micro-Kjeldahl method using a vacuum distillation. For N.P.N. we used the Folin-Wu tungstic acid filtrate.

Sodium was determined by the gravimetric method of Butler and

Tuthill (1931). Potassium was determined by modification of the method of Shohl and Bennett (1928) developed by E. Hartzler. Blood "sugar" was estimated by Somogyi's modification of the method of Shaffer and Hartmann (Peters and Van Slyke, 1931, p. 469).

Except for some of the sugar estimations and a few of the sodium analyses, all determinations were done in duplicate.

In a few cases cell volumes were measured by hematocrit, using a centrifuge cooled to 5°C. In other instances hemoglobin was estimated by the "photelometer" method of Sanford and Sheard (1930).

RESULTS. In agreement with previous studies, cell volume and hemoglobin measurements, where made, indicated increases up to 20 to 40 per cent in the concentration of hemoglobin in the cell. The principal results, in man, in regard to the constituents of the serum are summarized in table 1.

With the method used for blood "sugar," the equivalent of about 20 to 30 mgm. glucose represents other reducing substances—glutathione, ascorbic acid, etc. It is apparent from table 1 that in most of the cases the true blood glucose level must have fallen to near the vanishing point. It is notable that subject *D*, who was clinically very resistant to insulin, maintained his blood sugar significantly above zero in spite of the extremely large insulin dosages he received.

In all cases the insulin provoked a small increase in the concentration of proteins in the serum. This is in agreement with our findings in other series of similar cases in which only proteins and hemoglobin were studied. Elsewhere (Butt and Keys, 1938) we have given reasons why it seems probable that this small change in protein concentration represents simply the result of a loss of a small amount of fluid from the blood.

In all but one case the concentration of sodium rose slightly in the serum, but the change was so small as to be of negligible biological importance. Harrop and Benedict (1924), using less precise methods, found the serum sodium usually decreased slightly in rabbits and diabetic patients who received moderately large doses of insulin.

With a single exception, the non-protein nitrogen level in the serum decreased and this change, averaging 12.4 per cent, seems to be significant. Brunton (1925) stated that insulin is without effect on the N.P.N. level in the plasma in non-diabetics, a view supported by Lauter and Jenke (1925) from values for urinary nitrogen excretion. Luck and collaborators (Luck, Morrison and Wilbur, 1925; Kiech and Luch, 1928) reported a fall in the total amino acids in rats following the injection of insulin with a nearly equal increase in urea, but Peters and Van Slyke (1931) concluded that insulin appears to have no specific effect on nitrogen metabolism of normal individuals. In several of our cases plasma urea was analyzed separately; decreases of 1, 1.5 and 3 mgm. and an increase of 2 mgm. urea

TABLE 1

Changes in the concentrations of some of the blood constituents in man following the intramuscular injection of large doses of insulin

Time in minutes after injection; *R* indicates blood sample taken immediately before insulin

SUBJECT	TIME	UNITS OF INSULIN	BLOOD REDUCING SUBSTANCE AS SUGAR	TOTAL SERUM PROTEIN	CHANGE SERUM PROTEIN	SERUM N.P.N.	CHANGE N.P.N.	SERUM Na	CHANGE Na	SERUM K	CHANGE K
			mgm. per 100 cc.	*grams per 100 cc.*	*per cent*	*mgm. per 100 cc.*	*per cent*	*m.eq. per l.*	*per cent*	*m.eq. per l.*	*per cent*
A	R	90	99	6.20		16.5		144		4.82	
	72		22	6.39	+3.1	15.8	−4.2	144	0	4.20	−12.9
	98		37					133	−7.6	4.04	−16.2
B	R	60		7.04		15.2		137		6.16	
	73		33	7.41	+5.3	12.3	−19.1	142	+3.6	4.75	−22.9
C	R	70		6.80		19.1		136		5.03	
	75		22	6.90	+1.5	20.7	+8.4	138	+1.5	4.39	−12.7
D	R	130	104	6.86		19.5		137		4.90	
	42		44	7.18	+4.7	15.4	−21.0	138	+0.7	4.06	−17.1
	82		62					148	+8.0	5.11	+4.3
E	R	100		7.03		15.6		137		4.97	
	71		31	7.51	+6.7	14.3	−8.3	142	+3.7	3.80	−23.5
	94			7.05	+0.3	13.6	−12.8	141	+2.9	3.81	−23.4
A_1	R	60	108	6.32		18.7		144		5.26	
	98		39	6.48	+2.5	15.9	−15.0	145	+0.7	4.10	−22.1
F	R	20	110	6.72		26.4		143		4.93	
	85		83	6.79	+1.0	23.0	−12.9	145	+1.4		
	124		89					142	−0.7	4.14	−16.0
C_1	R	60	100	6.73		24.3		139		5.18	
	97		39	6.85	+1.8	22.2	−8.6	139	0	4.07	−21.4
D_1	R	120	110	6.57		24.3		138		5.21	
	71		50	7.25	+10.4	17.4	−28.4	141	+2.2	4.08	−21.7
	125		73					146	+5.8	4.78	−9.2
E_1	R	70	111	7.02		23.4				5.63	
	60†		40	7.67	+9.3	19.9	−15.0	138		4.12	−26.8
	65*							140		4.73	−16.0
	108							141		3.64	−35.3
Mean changes					+4.2		−12.4		+1.9		−18.4

† Sample taken during violent convulsions.

* Sample taken 5 minutes after intravenous injection of 20 cc. 50 per cent glucose solution.

N were found following insulin. Apparently other substances than urea accounted for much of the fall in N.P.N. It is notable that all the values for N.P.N. were very low; this may have been a result of the institutional diet. Rosenberg (1938) has reported a fall in the level of blood uric acid following massive doses of insulin.

There was a striking and consistent decrease in the concentration of potassium in the serum following insulin. In the samples taken at the height of the insulin reaction the change in serum potassium ranged from −12.7 to −26.8 per cent, with an average decrease of 18.4 per cent. A decrease in the concentration of potassium in the serum was observed in rabbits and in human diabetics following insulin by Harrop and Benedict (1924) and in dogs by Briggs and his colleagues (1924). Takeuchi (1928)

TABLE 2

Changes in the concentrations of some of the constituents in the blood in dogs following the intravenous administration of 45 units of insulin

Post insulin blood samples taken 60 minutes after the injection of insulin

DOG WEIGHT	BLOOD SAMPLE	BLOOD REDUCING SUBSTANCE AS SUGAR	SERUM PROTEIN	CHANGE SERUM PROTEIN	SERUM N.P.N.	CHANGE SERUM N.P.N.	SERUM Na	CHANGE SERUM Na	SERUM K	CHANGE SERUM K
kgm.		*mgm. per 100 cc.*	*grams per 100 cc.*	*per cent*	*mgm. per 100 cc.*	*per cent*	*m.eq. per l.*	*per cent*	*m.eq. per l.*	*per cent*
22	Before	98	5.96		36.8		158		4.60	
	After	49	6.71	+12.6	29.0	−21.2	165	+4.4	3.73	−18.9
19	Before	84	6.35		28.9		155		4.77	
	After	36	6.82	+7.4	25.1	−16.6	158	+1.9	4.02	−15.7

confirmed the finding of Harrop and Benedict that the urinary potassium output is elevated after insulin medication.

Kerr (1928) confirmed the observations of Briggs and colleagues on dogs and added the information that the potassium level in the erythrocytes of the dog is relatively unaffected by insulin. We have been able to confirm this last point by analyses of the red cells in three of our human subjects.

For comparison, studies were made on two laboratory trained normal dogs. The results are summarized in table 2. The responses of the dogs to insulin were closely similar to those seen in the human subjects—moderate rise in serum proteins, very small rise in sodium, and pronounced drops in the concentrations of non-protein nitrogen and of potassium in the serum.

Discussion. Peters and Van Slyke (1931, p. 787) state, with reference to the reported decrease of the serum potassium concentration following insulin, "the mechanism of the effect is unknown." At least a partial explanation seems to be available, however, in the recent finding that, in man, epinephrine produces a marked drop in the concentration of potassium in the serum (Keys, 1937, 1938). The suggestion of Wilder and Boothby (1923) that insulin causes a liberation of epinephrine into the blood stream has been supported by a long chain of evidence which seems to be conclusive (cf., for recent references, Saiszo, 1936; Marconi and Marco, 1937; Yen, Aomura and Inaba, 1933).

The magnitude of the decrease in serum potassium concentration reported here is significantly larger than usually results from a single intravenous injection of as much epinephrine as seems safe to use in man (up to 0.3 mgm. epinephrine chloride). However, we have observed decreases of serum potassium in man as large as 25 per cent following a single injection of epinephrine. It is well known that epinephrine is rapidly destroyed in the blood stream and it might be that considerably greater changes in serum potassium would result from the maintenance of a high level of epinephrine in the blood stream for a longer period of time. To test this point we gave five intravenous injections of 0.1 mgm. each to a normal man over a period of 40 minutes; five minutes after the last epinephrine injection the serum potassium concentration had fallen to 3.66 m.Eq. per liter as compared with the initial level of 5.20 m.Eq.; this is a decrease of 29.6 per cent.

A more serious question is raised by the fact that dogs do not differ from man in the response of the serum potassium to insulin. Ordinarily, dogs, as contrasted with man, exhibit a rise in the serum potassium in response to epinephrine. No explanation is offered for this apparent paradox.

The study on subject E_1 offers special evidence for the rôle of the adrenals in the reduction in serum potassium in insulin hypoglycemia. Five minutes after a temporary elevation of the blood sugar level by glucose injection, the serum potassium was much less decreased than in the acute hypoglycemic state both before and after the glucose injection. LaBarre and Sarie (1937) have shown that epinephrine liberation from the adrenals is a prompt response to hypoglycemia and that the secretion is suppressed at once when the sugar level is restored. We have shown that the potassium response to epinephrine in man is also very rapid, so that the potassium changes in subject E_1 are entirely consonant with the theory that the effect of insulin on the serum potassium is an indirect result of stimulation of the adrenals.

SUMMARY

Physically normal adult males with schizophrenia were injected with 20 to 130 units of insulin. In all cases (10 studies) the following changes in the composition of the blood serum were observed at the height of the insulin reaction:

1. A moderate increase in the concentration of the proteins, range 1.5 to 10.4 per cent, mean 4.2 per cent.

2. A marked decrease in the concentration of potassium, range 12.7 to 26.8 per cent, mean 18.4 per cent.

In all but one case the concentration of non-protein nitrogen in the serum decreased significantly; the average decrease was 12.4 per cent. At least a part of this change involved substances other than urea.

In all but one case the concentration of sodium in the serum rose slightly; the average increase was 1.9 per cent.

Studies on dogs yielded results very similar to those obtained on man.

The changes in potassium concentration in man are ascribed in large part to hyperactivity of the adrenal glands provoked by the hypoglycemia.

Acknowledgment. I wish to express my gratitude to Dr. B. F. Smith for facilities at the Rochester State Hospital, and to Drs. H. R. Butt and E. F. Rosenberg for assistance.

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PROLIFERATION OF CROP-SAC EPITHELIUM IN INCUBATING AND IN PROLACTIN-INJECTED PIGEONS STUDIED WITH THE COLCHICINE METHOD

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Received for publication May 3, 1938

The use of colchicine as a means of showing hyperplasia of animal tissues has been well established by others (1, 2, 3). In the present study the number of cells of crop-sac epithelium arrested in mitoses by colchicine is used as a measure of prolactin-induced proliferation (forms A and B) in that tissue. After finding the optimum colchicine dosage for this animal we have used this method to measure the speed and duration of the action of (A) a single intramuscular injection of prolactin on crop epithelium, together with an observation of the effect of superimposing a second injection of prolactin 8 hours after a first injection. We have also examined (B) the related and theoretically interesting problem of actual time relationships of the beginning of normal incubation behavior in pigeons and the beginning of an increased release of prolactin from the pigeon's own pituitary. Since an increase of crop-sac mitoses in the uninjected pigeons used in these latter tests satisfactorily indicates such a release of prolactin we were here able to obtain a wholly new test of the rôle of prolactin in the production of broodiness (4) and maternal behavior (5) in animals. An abstract of these results has been published (6).

RESULTS. For birds of 400–500 grams, adequately dosed with prolactin, 0.6 mgm. of colchicine (optimum dose) was found sufficient to arrest all dividing cells and yet low enough to permit all such cells eventually to complete their mitoses. Thereafter, 0.6 mgm. of colchicine and 60 units of prolactin, both injected into pectoral muscles, were used in all tests. In most cases (problem A) each bird yielded four samples of crop epithelium for histological examination. Three small pieces of tissue were excised at intervals of one-half hour, or of 2 hours, and a fourth sample was taken at a final period when the bird was killed. The time range of effectiveness of colchicine was determined by studying tissues taken when the prolactin effect was most pronounced—namely, at 2 to 6 hours after injection—but with the period after colchicine injection varying from 2 to 24 hours. The

colchicine dosage used was not fully effective for periods shorter than 6 hours, but between 6 and 16–18 hours it successfully arrested the dividing cells.

The prolactin-induced mitoses described here (problems A and B) were all observed within the time range at which colchicine was fully effective. After subcutaneous injections of prolactin Leblond and Allen (7) reported mitoses in the crop-sac at 36 hours.

Figure 1 records results obtained at various intervals following intramuscular injections of prolactin. Each plotted value represents the average number of mitoses found in 5 of the most stimulated high magnification fields in each of the several (exact number is stated in the white squares) epithelia examined at the various periods after injection. The control

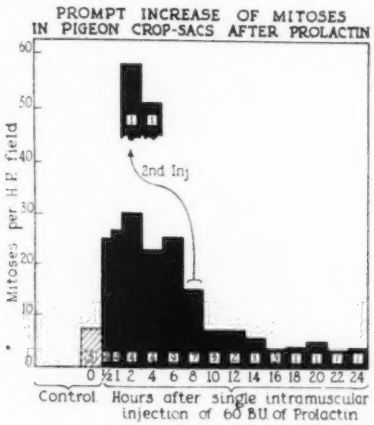

Fig. 1

values are low (av. = 7); the birds which supplied these data were sexually mature, taken in resting phases (not incubating or feeding young) of the reproductive cycle. The rapid absorption of prolactin is indicated by the very prompt response seen, at 0.5, 1 and 2 hours after injection. The very great increase of mitotic rate observed at 30 minutes suggests that at least traces of prolactin enter the circulation immediately after such injection, and that the stimulus to the division of some cells is also immediate. Maximum stimulation was obtained after 2 hours. After 6–8 hours the number of mitoses sharply diminished. However, a second injection of prolactin made at this 8-hour stage gave—at 2 and 4 hours later—a much greater number (58 against 30; 51 against 23) of mitoses than did the initial injection after the same intervals. This result is in full accord with the well established fact that a particular quantity of prolactin injected intra-

muscularly in a divided dose (three times daily; 8) produces a much larger crop-sac than when injected in a single daily dose.

The relation of crop-sac mitoses in uninjected pigeons to the onset and persistence of incubation behavior is next considered. According to Beams and Meyer (9) ordinary histological examination can first demonstrate proliferation or changes in the crop-sac at about the eighth day of incubation. Between the eighth and eighteenth days the proliferating areas of the crop walls are thrown into folds and enlarged approximately 20 times. The colchicine technique shows (fig. 2) that the mitotic rate is very low (av. = 4 in 4 tests) at and during the 3 days preceding the laying of the first egg. But 48 hours later, at the time the second egg is laid, a markedly increased mitotic rate (av. = 32) has become established. The significance of this observation is provided by the fact that it is precisely within this same 48-hour interval—and usually within less than 24 hours of the time

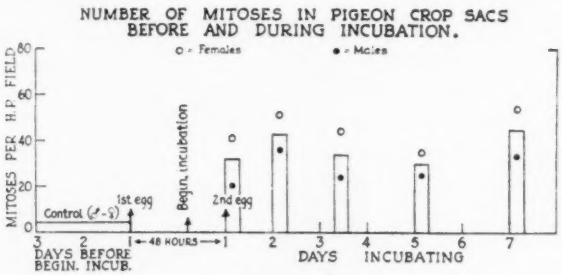

Fig. 2

the second egg is laid—that incubation behavior begins in both male and female pigeons. This increased rate (10 tests), or a still higher rate, is thereafter known to be maintained (numerous observations) throughout the broody period and also during the earlier days of feeding the young. We thus obtain definite evidence that a release of prolactin from the bird's own pituitary closely coincides with the time at which normal broodiness is initiated in these animals.

The 8 illustrations of figure 3 supply specific items of evidence supporting the conclusion just stated. In all cases cell division is restricted to the basal or deep-lying cells of the mucosa; and this area is at or near the bottom or lower surface of each specimen shown. The dividing cells are large clear cells with quite large nuclei, these latter representing chromosomes typically clumped at the metaphase. "Controls," A and E, represent the extremes found in single high magnification fields; in A there are no mitoses; in E there are several mitoses, and this particular photograph shows the most stimulated area found in any section of any of the several crop-sacs examined in the control period (0–3 days before laying of the

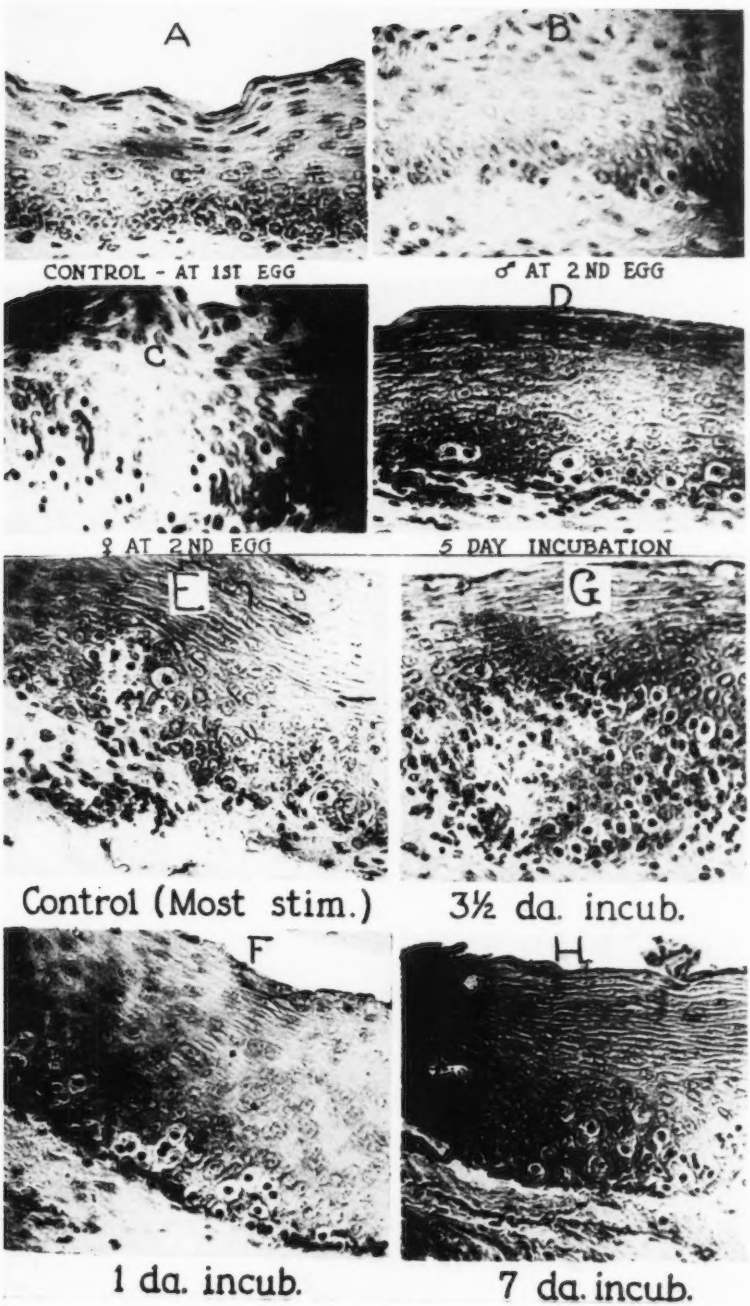
A
B
CONTROL - AT 1ST EGG
♂ AT 2ND EGG
C
D
♀ AT 2ND EGG
5 DAY INCUBATION
E
G
Control (Most stim.)
3½ da. incub.
F
H
1 da. incub.
7 da. incub.

Fig. 3

"first" egg). Forty-eight hours after the first egg is laid, and coincident with the laying of the second egg (B, C), dividing cells are relatively numerous; dividing cells are also relatively numerous at 1 to 7 days of incubation (F, G, D, H). Ordinary histological methods have earlier clearly shown that still later phases of the broody period are accompanied by rapid cellular proliferation in the crop-sacs.

On figure 2 the dots and circles indicate the number of mitoses found at 1–7 days of incubation in males and females, respectively. This number was markedly smaller in males than in females in all of the 5 periods studied, and this is believed to indicate a real or true variable related to sex. Possibly this difference reflects, or accounts for, the common observation that male pigeons usually show somewhat less devotion to eggs and nests than do females; again, perhaps some importance attaches to the fact that both sexes seem less devoted to eggs during the first days of incubation (when their crop-sacs are proliferating less actively and less prolactin is released to their blood) than during the last half of the incubation cycle.

Discussion. The coincidence of an increased output of prolactin from the pigeon's own pituitary with the onset of broodiness in that animal seems important. In this case only normal or physiological quantities of the hormone are involved, and here these quantities enter the circulation in a wholly normal way. Earlier studies on the relation of prolactin to broodiness in fowl (4) and maternal behavior in rats (5) have involved the *injection* of prolactin, and indeed in quantities which had a rather marked antigonad action (10, 11) besides still other obvious effects. The present study therefore supplies evidence from another species, and also evidence of another type, in support of the conclusion that prolactin is causally involved in the cyclic production of broody and parental behavior in animals.

SUMMARY

Apparently all mitoses in the crop-sac epithelium of adult pigeons weighing 450 grams are arrested in metaphase from the sixth to the sixteenth hour after intramuscular injection of 0.6 milligram of colchicine.

With the aid of such colchicine treatment an increased proliferation of the crop-sac is observed to follow promptly the intramuscular injection of 60 units of prolactin. This increased proliferation attains its maximum after 2 hours, and is no longer observable after 10–12 hours. A second injection of prolactin made 8 hours after an initial injection produced, at 2 and 4 hours later, a much higher mitotic rate than that which followed the first injection.

The employment of this same colchicine technique in pigeons immediately preceding and following the initiation of broody behavior has indicated a close coincidence of increased release of prolactin from the bird's

own pituitary and the time of onset of this incubation (broody) behavior. At one day after the beginning of broodiness the preceding normal or repair rate of mitosis in the crop-sac has already increased by approximately 600 per cent. This new rate is fully maintained during the first half of the incubation period, and is known to be still further increased during the last half of incubation.

These results give further support to the conclusion earlier stated by Riddle, Bates and Lahr concerning a significant rôle of prolactin in the cyclic development of broody and maternal behavior in higher animals.

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WORK PERFORMANCE OF HYPOPHYSECTOMIZED RATS TREATED WITH ANTERIOR PITUITARY EXTRACTS

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Received for publication May 4, 1938

After removal of the anterior lobe of the pituitary gland in the rat there is extensive atrophy of the cortex of the adrenal. Also following removal of the anterior pituitary lobe the capacity of skeletal muscle to sustain work when faradic stimulation is applied is greatly decreased (3). Treatment of the hypophysectomized rat with cortin has been shown partially to restore the capacity for work (4). One of us (Moon, 5) has described the separation of an adrenocorticotropic substance from anterior pituitary extracts which is almost completely free from the other known principles occurring in the pituitary body. The adrenocorticotropic principle has the property of either preventing the atrophy of the adrenal cortex which occurs following hypophysectomy, or of restoring the cortex after atrophy has once occurred.

The primary object of this study was to determine the effect on work of treating hypophysectomized rats with the adrenocorticotropic extract when the adrenal glands were intact and when they had been removed. We have also compared the work performance of these animals with similar animals treated with the growth principle, the mammotropic principle, and the thyrotropic principle.

METHODS. Male rats of the Wistar strain, which were uniform as to age and which weighed approximately 180 grams, were used in these experiments. The pituitary body was completely removed by the usual ventral parapharyngeal approach. Those animals which had either hemorrhage or dyspnea as a result of the operation, and animals about which there was any doubt regarding the completeness of the operation, were immediately discarded. The work tests were uniformly begun seven days after operation.

Following operation the animals were maintained on a commercial diet containing 0.24 per cent sodium and 1 per cent potassium and they were given a 4 per cent solution of glucose to drink. When cortin was administered it was added to the drinking water and the pituitary extracts were administered once daily by intraperitoneal injection. All injections were continued after the beginning of the work tests.

In the work tests, the animals were anesthetized with phenobarbital sodium. The gastrocnemius muscle was loaded with 100 grams and was made to contract three times per second by direct faradic stimulation. The details of this procedure have been previously described (1). The initial rate of work was recorded and the animals were observed at appropriate intervals until the stimulated muscle lost responsiveness. All of the animals received 1 cc. of a 20 per cent solution of glucose intraperitoneally at the beginning of the work test and at each subsequent twelve-hour interval.

The cortin used in these experiments had been previously assayed by the Ingle (2) rat test. The optimal effect on the work performance of adrenalectomized rats was obtained with 0.5 cc. of the solution administered at each twelve-hour interval.

RESULTS. In experiment 1, the work performance of ten untreated hypophysectomized rats was studied one week after the operation. The initial rates of work were greatly decreased below normal and the work totals were very small. The work records for this group compare favorably to values obtained in previous experiments.

In experiment 2, ten rats were treated daily with 2 mgm. of the growth principle. Ten additional rats were treated daily with 4 mgm. This extract had a marked effect in preventing loss in body weight subsequent to hypophysectomy. The initial rates of work and the work totals were definitely increased above the values for the untreated animals. Atrophy of the adrenal glands was not so marked as that occurring in the untreated animals.

In experiment 3, twelve rats were treated daily with 2 mgm. of the mammotropic principle. Twelve additional rats were treated daily with 4 mgm. The averages for the initial rates of work and the work totals were increased above the levels for untreated animals. There was a tendency for treatment with this extract to retard the loss of body weight following operation. Atrophy of the adrenal glands was less in this group than in the group of untreated animals.

In experiment 4, eleven rats were treated daily with 2 mgm. of the thyrotropic principle. Eleven additional animals were treated daily with 5 mgm. The effects on body weight and work performance were again positive. The weights of the thyroid gland were all within the range of normal variability. Atrophy of the adrenal glands was not as marked in this group as in the group of untreated animals.

In experiment 5, twelve rats were treated daily with 2 mgm. of the adrenocorticotropic principle. The values for the initial rates of work approached normal and the values for total work were greatly increased over the values for untreated animals. There was some tendency for treatment with this extract to retard the loss in body weight following

operation. The weights for the adrenal glands were all within the limits of normal variability and the average value compared favorably with the average value for adrenal weights in normal rats.

In experiment 6, the pituitary body and the adrenal glands were removed from twenty rats in a single stage operation. Immediately after operation each rat received 2 cc. of cortin by subcutaneous injection. Treatment with cortin was then continued by administering 2 cc. to each rat daily in the drinking water. Ten of the rats were given 2 mgm. of the adrenocorticotropic principle daily. The ten remaining animals did not receive

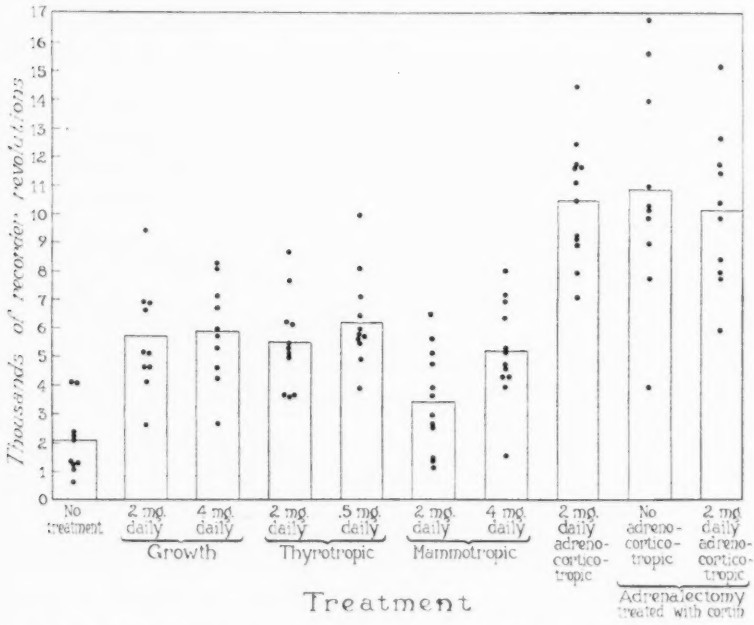

Fig. 1. The work performance of hypophysectomized rats treated with anterior pituitary extracts.

any additional treatment. At the beginning of the work test, and at each subsequent twelve-hour interval each rat received 1 cc. of cortin by subcutaneous injection. The work performance of the animals in this experiment compared favorably to that of the animals in experiment 5, and also to the values previously published for cortin treated hypophysectomized rats (4). There was no apparent effect on work following the administration of adrenocorticotropic principle when the adrenal glands were absent; however, there was a definite effect on body weight following the administration of adrenocorticotropic principle to these animals.

The group averages and individual values for total work are summarized in figure 1. Other data are summarized in table 1. In a previous experiment (3) the average work performance of normal rats of comparable weight was found to be 67,711 recorder revolutions. This value is far greater than any of the individual performances recorded in the present experiment.

COMMENT. The principal morphologic effect of administering the adrenocorticotropic principle to hypophysectomized rats is in maintaining the adrenal cortex. Since it has been demonstrated that cortin has a definite effect in enhancing the work performance of hypophysectomized

TABLE 1

Work performance of hypophysectomized rats

AMOUNT OF PRINCIPLE ADMINISTERED DAILY*	NUMBER	FINAL BODY WEIGHT		INITIAL RATE† OF WORK		ADRENAL WEIGHT	
		Average	Range	Average	Range	Average	Range
		grams	*grams*			*mgm.*	*mgm.*
Untreated	10	143	134–151	8.2	4–11	16.3	13–19
Growth—2 mgm.	10	178	160–202	12.3	8–16	18.6	14–25
Growth—4 mgm.	10	178	162–192	12.5	10–16	20.4	16–24
Mammotropic—2 mgm.	12	139	130–149	9.9	6–13	16.2	14–19
Mammotropic—4 mgm.	12	145	137–153	10.6	7–13	19.2	16–25
Thyrotropic—2 mgm.	11	155	145–165	13.1	10–16	20.0	18–23
Thyrotropic—5 mgm.	11	154	141–162	10.8	9–13	19.2	16–22
Adrenocorticotropic—2 mgm.	12	157	148–165	15.3	12–19	27.3	22–32
Cortin only (hypophysectomy plus adrenalectomy)	10	136	125–142	16.1	13–19		
Cortin plus 2 mgm. adrenocorticotropic principle (hypophysectomy plus adrenalectomy)	10	148	140–158	15.6	12–20		

* Expressed as amount of solids.

† Expressed as recorder revolutions. Each revolution represents approximately 400 gram-centimeters of work.

rats, and that the adrenocorticotropic principle has a similar effect on work when the adrenal glands are present but no additional effect when they are absent, it must be concluded that the adrenocorticotropic principle of the anterior pituitary lobe has the property of increasing the output of cortin from the adrenal cortex.

Perla (6, 7) has described an increase in the resistance of the hypophysectomized rat to histamine when treated with cortin and when treated with anterior pituitary extracts containing the adrenotropic principle.

It may be anticipated that any principle secreted by the anterior lobe of the pituitary gland will have some effect on the muscular asthenia of the

hypophysectomized animal. It was of interest to note that treatment of our animals with a highly active preparation of the growth principle had only a small effect on work performance. Since other preparations contained only small amounts of the adrenocorticotropic principle it is possible that their effects would be more apparent in animals in which the adrenal insufficiency had been corrected. We have not yet conducted experiments in which we have studied the combined effects of anterior pituitary principles on the work performance of hypophysectomized animals.

SUMMARY

The work performance of hypophysectomized rats is greatly decreased. The work performance of hypophysectomized rats treated with growth, mammotropic and thyrotropic extracts is definitely improved. Hypophysectomized rats treated with adrenocorticotropic hormone show a greater improvement in work capacity than those treated with other pituitary extracts, and compare favorably with those treated with cortin. The work capacity of hypophysectomized, adrenalectomized rats maintained with cortin is not improved by the administration of adrenocorticotropic extracts. The work capacity of hypophysectomized rats treated with any of the anterior pituitary extracts is less than normal.

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THE RELATION OF INSULIN AND ADRENALIN TO URIC ACID EXCRETION IN THE RABBIT

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Received for publication May 9, 1938

The effect, in the human subject, of certain amino acids (1), of pyruvic acid (2) and of glucose (3) in increasing the urinary output of uric acid, and the ineffectiveness of fat, suggested the possibility of a stimulatory action exerted by an antiketogenic substance (4). This action might consist in a lowering of the kidney threshold, which is elevated in fasting, or in an actual increase in the rate of purine catabolism.

Among the hormones directly concerned in the metabolism of carbohydrate, insulin, according to Larson and Chaikoff (5), increased the output of allantoin in dogs; in the Dalmatian (6), the administration of insulin increased the uric acid in both blood and urine but did not affect the excretion of allantoin. They concluded that insulin was not directly responsible since the fall in blood sugar produced by it preceded the rise in uric acid and the latter could be prevented by simultaneous administration of glucose and insulin. They (7) found, furthermore, that administration of adrenalin to ordinary dogs also increased allantoin excretion and in Dalmatians there was a prompt response in increased blood and urinary uric acid. The rise in uric acid in the blood was more prompt and more pronounced than that of glucose. A secretion of adrenalin, evoked by the insulin hypoglycemia, was therefore the more probable mechanism of the stimulating action of insulin.

The purpose of these experiments was to determine the effects of these hormones in another species, the rabbit, in which the excretion of uric acid is not inconsiderable in proportion to body weight.

EXPERIMENTAL. After a diet of oats and alfalfa rabbits were fasted 20 hours. Thereafter, in 2-hour periods, the urine was obtained by gentle pressure over the urinary bladder. The hormone preparations were administered at the end of the first two hour period. Sterile glucose solution was injected if unmistakable signs of convulsions appeared following insulin. When blood was to be analyzed, 5 cc. samples were drawn from the marginal ear vein.

For the determination of uric acid, this was first precipitated as the zinc salt by the Morris and MacLeod reagent (8) and the color was developed

by the Folin procedure (9). Gibson's micromethod (10) was used for the determination of blood sugar, Folin's method for determining creatinine (11) and the Koch and McMeekin methods (12) for urinary total nitrogen and blood non-protein nitrogen.

RESULTS. Typical effects of the injection of adrenalin and insulin on the uric acid excretion of rabbits appear in table 1. The three control experiments show a fairly constant level of uric acid excretion, especially

TABLE 1

Excretion of uric acid by the rabbit after the injection of insulin and adrenalin

ANIMAL		URIC ACID EXCRETION IN 2-HOURLY PERIODS					SUBSTANCE* INJECTED
Number	Weight	First	Second	Third	Fourth	Fifth	
	kgm.	*mgm./hr.*	*mgm./hr.*	*mgm./hr.*	*mgm./hr.*	*mgm./hr.*	
2	2.9	0.55	0.87	0.81	0.81		2 cc. Ringer's solution
12	2.9	0.70	0.49	0.56	0.63	0.52	2 cc. Ringer's solution
11	2.6	0.90	0.56	0.55	0.60	0.42	2 cc. Ringer's solution
A	3.3	0.43	0.46	0.49	0.48		0.05 mgm. adrenalin
D	2.6	0.57	1.13	0.86	0.89		0.26 mgm. adrenalin
C	3.0	0.30	0.63	0.48	0.49		0.30 mgm. adrenalin
12	3.1	0.33	1.90	1.20	0.25	0.21	0.62 mgm. adrenalin
5	3.1	0.36	2.20	1.80	0.59	0.50	0.62 mgm. adrenalin
4	2.3	0.52	0.63	0.81	0.74	0.58	1.0 unit insulin
5	2.3	0.55	0.80	0.76	0.49	0.58	1.5 units insulin
5	2.7	0.32	0.57	1.29	0.52	0.52‡	4.0 units insulin
4	2.3	0.50	0.11	1.98†	2.36	1.14	4.0 units insulin
2	2.5	0.82	0.40	1.04	0.56†	0.56‡	4.0 units insulin
5	2.4	0.42	0.87	1.13	0.50	0.50‡	4.0 units insulin
9	3.0	0.57	0.61	1.25†	0.62	0.62‡	4.0 units insulin
F		0.45	0.50	0.25	0.20		4.0 units insulin preceded (45 min.) by 40 grams glucose by mouth

* Injected at the end of the first period.

† Glucose injected.

‡ Four-hour collection period.

during the last three collection periods, after any earlier excitement of the animals might have subsided. Administration of small amounts of adrenalin had no effect on uric acid excretion. With larger amounts the excretion was increased immediately following the hormone injections.

The injection of small amounts of insulin was followed by insignificant increases in uric acid excretion. Larger doses caused noticeable increases beginning after two to four hours. This delayed response may reasonably

be attributed to the time required for the hypoglycemia to cause an increased secretion of adrenalin. When glucose was administered prior to insulin, uric acid excretion was not affected; blood glucose was not lowered sufficiently to cause the secretion of adrenalin.

Parallel hourly determinations of uric acid in the blood were made in a number of instances in the hope that the figures would indicate whether there was a change in kidney threshold or, as in the case of the Dalmatian, an increased production of uric acid and a higher level in the blood as well

TABLE 2

The excretion of total nitrogen, creatinine, and uric acid after the injection of adrenalin

	TIME	TOTAL N	CREATININE	URIC ACID	C/N*	U/N†	REMARKS
		mgm. per hour	*mgm. per hour*	*mgm. per hour*			
Rabbit D	6 to 8	37	4.1	0.35	0.111	0.0095	
	8 to 10	49	4.6	0.41	0.094	0.0083	2 units insulin at 8:40
	10 to 12	97	8.6	0.73	0.088	0.0075	
	12 to 2	52	3.8	0.41	0.073	0.0079	0.25 mgm. adrenalin at 10:20
	2 to 4	53	4.3	0.48	0.081	0.0090	
	4 to 6	58	5.0	0.51	0.086	0.0088	
Rabbit C	6 to 8	60	5.9	0.64	0.098	0.0108	3 units insulin at 8:00
	8 to 10	67	6.2	0.70	0.093	0.0104	
	10 to 12	74	6.5	1.07	0.088	0.0145	0.3 mgm. adrenalin at 10:10
	12 to 2	60	5.0	0.76	0.083	0.0126	Glucose at 11:10
	2 to 4	63	5.9	0.79	0.094	0.0125	

* C/N is the ratio of creatinine to total nitrogen.

† U/N is the ratio of uric acid to total nitrogen.

as in the urine. The amounts found after the larger doses of adrenalin and insulin (delayed) suggested the latter explanation, but as the highest figures obtained were only 0.50 to 0.75 mgm. per 100 cc. they do not justify any conclusions.

To determine to what extent the changes in the excretion of uric acid resulting from insulin and adrenalin injections might be attributed to a general stimulatory action on protein metabolism, insulin was injected into experimental animals and a short time later, adrenalin was injected to prevent too severe lowering of blood sugar. Uric acid, creatinine and

total nitrogen were determined in two hour intervals. To reveal the relative changes in each constituent the ratio of creatinine to total nitrogen (C/N) and that of uric acid to total nitrogen (U/N) were calculated. It may be seen from typical results in table 2 that the rate of excretion of creatinine was unaffected while that of uric acid, particularly with 0.3 mgm. adrenalin, increased. This increase was usually accompanied by a relatively smaller increase in the total nitrogen of the urine.

In the experiments of Larson and Chaikoff (5) a 25 to 50 per cent increase in urea nitrogen accompanied the approximately 100 per cent increase in allantoin excretion during the five hours following insulin administration. Miyahara's (13) experiments demonstrated a marked increase (300 per cent and more) in the daily output of uric acid by rabbits receiving a single dose of 0.15 mgm. adrenalin; allantoin excretion also rose but much less (60 per cent), and total nitrogen still less (15–30 per cent). A rise of 10 to 30 per cent in hourly creatinine excretion after 0.5 mgm. of adrenalin was reported by Kaziro and Taku (14); they used no smaller doses. On the other hand Palladin and Tichwinskaja (15) found that gradually increasing daily doses of adrenalin, up to as much as 6 mgm., had no effect on the daily output of creatinine or total nitrogen; doses of 2 mgm. twice daily produced an increase in both.

It is difficult to correlate all of these results, but it would appear that adrenalin, physiologically produced or administered by injection, not only affects the metabolism of purines but also exerts a general increase in cellular activity and protein catabolism. Species differences are probably not negligible factors. In experiments not further recorded here the administration of 30 units of insulin to a normal human subject and of 40 units to 3 diabetic subjects, although lowering the blood sugar, produced no significant changes in the uric acid content of the blood or the urine. The injection of 0.5 or 1.0 mgm. of adrenalin in three normal subjects also produced no significant changes in the excretion of uric acid.

SUMMARY

The injection of insulin or adrenalin into rabbits increases the amount of uric acid excreted. Insulin exerts its effect indirectly by lowering the blood sugar and causing mobilization of adrenalin.

Adrenalin appears to affect purine metabolism first but protein catabolism as a whole may also be stimulated.

In the human organism insulin and adrenalin administered in therapeutic amounts, have no significant effect on the rate of excretion of uric acid.

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RENAL FUNCTION IN THE OPOSSUM AND THE MECHANISM OF CORTICO-ADRENAL AND POST-PITUITARY ACTION[1]

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Received for publication May 11, 1938

In earlier papers in which we have described some of the effects of adrenalectomy on renal excretion in the opossum, the possibility of an important interrelationship between the adrenal cortex and the posterior lobe of the hypophysis has been suggested (Silvette, 1937, 1938; Silvette and Britton, 1938). We have now tested the response of both normal and adrenalectomized opossums to orally administered water, salt and urea under the influence of cortico-adrenal extract, post-pituitary solution and adrenalin. This animal type has been shown to be peculiarly adapted to the study of kidney function because of the ease with which catheterization may be carried out and quantitative determinations of renal output made.

METHODS. Previous reports have been based on a consideration of the total 24-hour urinary output of the opossum under various conditions. This technique was advantageous in determining the renal effects of such substances as cortico-adrenal extract, which act over a relatively long period of time, but the same 24-hour metabolic period was found to be too long for evaluating the transient effects of post-pituitary solution and adrenalin. We have therefore varied our earlier technique as follows:

Female opossums were fasted for 18 hours, then given 2 per cent per kilo body weight of one or another solution by stomach tube every two hours. After a further period of 6 hours, during which the animals came into equilibrium under the experimental conditions, all the urine in the bladder was withdrawn and discarded. Urine specimens were thereafter collected by catheter for three consecutive 2-hour periods, and separately measured and analyzed for chlorides (Peters and Van Slyke, 1932, p. 833) and urea (Van Slyke and Kugel, 1933). Both normal and adrenalectomized animals were used. In different experiments they were given distilled water or 0.9 per cent sodium chloride solution or a solution containing 0.9 per cent sodium chloride plus 2 per cent urea. The effects of

[1] Grateful acknowledgment is made of aid received from the Rockefeller Foundation.

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cortico-adrenal extract (Ehrenstein and Britton, 1937), post-pituitary solution (Squibb) and adrenalin were then determined.

Cortico-adrenal extract was routinely administered by mouth to the amount of one per cent per kilo body weight every two hours, the first dose being given two hours before the collection of the first urine sample. Both post-pituitary solution (containing 20 international units per cubic centimeter) and adrenalin (1:1000) were given subcutaneously, the former in doses of 0.3 cc. per kilo every two hours and the latter in doses of 0.2 cc. per kilo every two hours. The initial injection was made two hours before the collection of the first urine sample. More than 30 animals were utilized in over 300 experiments during the study.

Results. Detailed analyses of the results obtained in the various experiments are given in tables 1, 2 and 3. Average figures and their probable errors, together with the differences between the hormone-treated series and their controls, divided by the probable errors of these differences, have been calculated (see note at bottom of table 1). When

$$\frac{\text{[difference between control and experimental series]}}{\text{Probable Error}_{\text{difference}}}$$

is greater than 3, the difference has been assumed to have statistical significance. There were, however, several series in which the quotients so calculated were somewhat below 3 (from 2.27 to 2.93), and in the discussion such differences were allowed a degree of significance, biologically if not precisely statistically.

The experimental results are clearly evident from the tables, and require only brief summation. Since cortico-adrenal extract sometimes contains small amounts of adrenalin, and post-pituitary solution also contains a pressor substance along with the antidiuretic hormone, it was thought well to include several control series in which adrenalin was injected. From the results adrenalin may be said to show no significant influence on the renal excretion of both normal and adrenalectomized opossums under the experimental conditions. Thus the effects of cortico-adrenal extract and post-pituitary solution on renal excretion (later to be noted) cannot be considered due to the presence of pressor substances.

The injection of post-pituitary solution, however, inhibits the diuresis produced by the ingestion of water, but is incapable of accelerating the reabsorption of water if the concentration of salts in the urine is very great, i.e., after the administration of salt or salt-urea solutions. These results were observed in both normal and adrenalectomized animals, and in the case of the unoperated opossums they confirm the experiences of other workers on different animal types (Motzfeldt, 1917). Injection of post-pituitary solution invariably led to an increase in the excretion of chlorides, both in concentration (per cubic centimeter of urine) and in absolute

amount; further, except in the case of urea administration, it produced a relative and absolute increase in urea excretion.

Administration of cortico-adrenal extract to normal animals, in contrast, led to variable but significant increases in water or total chloride excretion, while the total urea output remained unchanged. After adrenalectomy,

TABLE 1

Effect of cortico-adrenal extract, post-pituitary solution and adrenalin on renal output of normal and adrenalectomized opossums given distilled water by mouth

SERIES	NO. ANIMALS IN SERIES	NO. METABOLISM RUNS	OPERATION	HORMONE ADMINISTERED	URINARY EXCRETION PER KILO BODY WEIGHT PER 2 HOURS (AVERAGE FIGURES ±PROBABLE ERRORS)				
					Water	Chlorides		Urea	
						Per cc.	Total	Per cc.	Total
					cc.	*mgm.*	*mgm.*	*mgm.*	*mgm.*
A	5	15	None	None	16.4 ±0.87	0.21	3.4 ±0.36	4.51	74 ±6.2
B	4	12	None	Cortico-adrenal extract	17.6 ±1.22 (0.80)	0.32	5.7 ±0.70 (2.92)	3.58	63 ±4.8 (1.40)
C	4	12	None	Post-pituitary solution	5.7 ±0.58 (10.2)	6.50	37 ±4.3 (7.80)	18.2	104 ±6.6 (3.32)
D	4	12	None	Adrenalin	22.6 ±1.12 (4.38)	0.155	3.5 ±0.5 (0.16)	3.32	75 ±2.4 (0.15)
E	6	18	Adrex*	None	8.1 ±0.87	0.32	2.6 ±0.4	6.66	54 ±3.9
F	5	15	Adrex	Cortico-adrenal extract	16.9 ±1.07 (6.38)	0.30	5.1 ±0.4 (4.43)	4.91	83 ±5.6 (4.24)
G	5	15	Adrex	Post-pituitary solution	3.0 ±0.38 (5.38)	1.72	5.2 ±0.9 (2.64)	25.0	75 ±4.1 (3.71)
H	4	12	Adrex	Adrenalin	6.5 ±0.99 (1.21)	0.20	1.3 ±0.25 (1.46)	6.60	43 ±6.5 (1.46)

Figures in parentheses represent the differences between the hormone-treated series and their controls, divided by the probable errors of their differences:

$$\frac{\text{Difference between control and experimental}}{\text{P.E.}_{\text{difference}}}$$

where $\text{P.E.}_{\text{diff.}} = \pm \sqrt{[\text{P.E.}_{\text{cont.}}]^2 + [\text{P.E.}_{\text{exp.}}]^2}$

* Adrenalectomy.

however, the extract brought about significant increases in water, chloride and urea output in animals fed distilled water or sodium chloride solution. It is important to note that although salt and urea excretion was absolutely increased, yet per cubic centimeter of urine the amounts eliminated were usually much diminished. The one exception to this significant

observation (later to be discussed) was chloride excretion in animals given distilled water and cortico-adrenal extract (table 1, B and F).

It was also interesting to note that throughout the experiments the oral administration of cortico-adrenal extract was highly effective. As a comparative test of the general experimental series, one group of ad-

TABLE 2

Effect of cortico-adrenal extract, post-pituitary solution and adrenalin on renal output of normal and adrenalectomized opossums given 0.9 per cent sodium chloride by mouth

SERIES	NO. ANIMALS IN SERIES	NO. METABOLISM RUNS	OPERATION	MATERIAL ADMINISTERED	URINARY EXCRETION PER KILO BODY WEIGHT PER 2 HOURS (AVERAGE FIGURES ±PROBABLE ERRORS)				
					Water	Chlorides		Urea	
						Per cc.	Total	Per cc.	Total
					cc.	*mgm.*	*mgm.*	*mgm.*	*mgm.*
A	5	15	None	None	13.2 ±1.07	7.12	94 ± 5.3	6.51	86 ±5.8
B	7	21	None	Cortico-adrenal extract	22.0 ±1.41 (4.97)	6.37	140 ± 6.3 (5.60)	4.18	92 ±4.1 (0.85)
C	6	18	None	Post-pituitary solution	17.0 ±1.06 (2.79)	10.0	170 ±10.6 (6.41)	6.77	115 ±8.0 (2.93)
D	4	12	None	Adrenalin	16.1 ±2.44 (0.34)	5.65	91 ±13.4 (0.21)	5.90	95 ±7.9 (0.92)
E	6	18	Adrex†	None	5.2 ±0.54	7.50	39 ± 3.2	10.2	53 ±1.8
*	4	12	Adrex	Cortico-adrenal extract	9.0 ±1.19*	8.34	75 ± 6.6*	9.35	84 ±4.9*
F	7	21	Adrex	Cortico-adrenal extract	12.2 ±0.73 (7.69)	4.58	56 ± 6.8 (2.27)	7.62	93 ±7.3 (5.35)
G	4	12	Adrex	Post-pituitary solution	8.8 ±0.78 (3.79)	11.1	98 ±11.9 (4.79)	10.3	91 ±6.5 (5.65)
H	4	12	Adrex	Adrenalin	3.4 ±0.40 (2.69)	6.18	21 ± 1.9 (4.84)	16.5	56 ±7.5 (0.39)

Figures in parentheses represent the differences between hormone-treated series and their controls, divided by the probable error of these differences.

* Cortico-adrenal extract given intraperitoneally.

† Adrex.

renalectomized animals was given the same amount of extract intraperitoneally, and the results compared with those derived from orally-treated animals. The comparison would seem to indicate (table 2) that intraperitoneal injection is somewhat more efficacious.

Discussion. It may be emphasized that in the experiments herein

reported the administration of cortico-adrenal extract has been made orally as a routine measure (see Britton, Flippin and Silvette, 1931).

Most investigators have concluded that the antidiuretic hormone of the posterior lobe (or hypothalamic tissues) acts directly on the kidney, or more specifically on tubular reabsorption of water; and that it is without consistent effect on the rate of glomerular filtration (Burgess, Harvey and

TABLE 3

Effect of cortico-adrenal extract and post-pituitary solution on normal and adrenalectomized opossums given urea-sodium chloride solution by mouth

Each figure represents an average of 12 runs on 4 animals.

SERIES	OPERATION	MATERIAL ADMINISTERED	URINARY EXCRETION PER KILO BODY WEIGHT PER 2 HOURS (AVERAGE FIGURES ±PROBABLE ERRORS)				
			Water	Chlorides		Urea	
				Per cc.	Total	Per cc.	Total
			cc.	*mgm.*	*mgm.*	*mgm.*	*mgm.*
A	None	None	10.0 ±0.38	7.60	76 ± 3.1	40.6	406 ± 9.0
B	None	Cortico-adrenal extract	15.2 ±1.16 (4.26)	6.31	96 ±10.0 (1.92)	27.2	414 ±14.5 (0.47)
C	None	Post-pituitary solution	14.8 ±1.68 (2.79)	8.51	126 ±10.7 (4.50)	26.5	392 ±26.6 (0.50)
D	Adrex*	None	8.8 ±0.76	4.20	37 ± 3.9	38.6	340 ±17.0
E	Adrex	Cortico-adrenal extract	13.3 ±0.99 (3.60)	3.31	44 ± 4.1 (1.24)	33.3	443 ±23 (3.60)
F	Adrex	Post-pituitary† solution					

Figures in parentheses represent the differences between the hormone-treated series and their controls, divided by the probable error of these differences.

* Adrenalectomized.

† It may be mentioned that the presence of some smooth-muscle stimulating hormone in the post-pituitary solution used gave some trouble in quantitative collection of urine samples from the series in which this substance was used. Apparently bladder tonus was so greatly increased that it was sometimes impossible for animals to retain urine for even a 2-hour period. This series was lost in this way, for adrenalectomized animals given a solution of salt plus urea seemed particularly sensitive to the oxytocic effect of pituitary solution. Some observations indicate, however, that the total urine excreted in a 2-hour period was less than that of the controls.

Marshall, 1933; Peters, 1935; Smith, 1937). The hormone is also said to induce slight but variable increases in the excretion of chlorides (McIntyre and VanDyke, 1931; Peters, 1935).

Our own observations now show positively that when post-pituitary solution is injected into normal or adrenalectomized animals, either salt- or water-fed, there results an increased concentration of chlorides per

cubic centimeter of urine, as well as an increase in total excretion (tables 1, 2, 3). Injection of post-pituitary solution also increases the concentration and total output of urea (tables 1, 2), although this effect is not observed when, after 2 per cent urea administration, urea excretion is already at a maximum (table 3, C).

The opposite effect is observed, very strikingly, after administration of cortico-adrenal extract to animals under the same experimental conditions. In this case reduction in chloride concentration per cubic centimeter of urine is an almost invariable effect (see exception, table 1, B and F, and the following discussion), and is coupled with increased urinary output of water. The function of the antidiuretic hormone of the pituitary is to concentrate urine (or increase the reabsorption of water), but when the urine is already concentrated (as in table 2, C and G and table 3, C) this hormone is without effect. The action of the cortico-adrenal hormone is, on the contrary, to dilute the urine (by decreasing tubular reabsorption of water), and by analogy it should be without effect when the urine is already very dilute. Our evidence demonstrates that the latter is the case in actual experiment: when distilled water is given by mouth to normal animals, a very dilute urine is secreted (table 1, A); and addition of cortico-adrenal extract to animals so treated is without further urine-diluting effect (table 1, B and F).

It seems proper to emphasize the changes in concentration rather than total excretion since conclusions drawn from a consideration of total output may be misleading. Thus, both post-pituitary solution and cortico-adrenal extract lead to increased total output of chlorides (table 2); but examination of the data shows that the increases were not due to similar causes. After pituitary administration a small increase in urine output coupled with a great increase in chloride concentration per cubic centimeter resulted in increased chloride output during the metabolic period; after cortico-adrenal extract a large increase in fluid output was accompanied by a greater chloride output than in the controls, although in this case chloride concentration per cubic centimeter was definitely reduced. Every form of diuresis, it should be noted, tends to sweep considerable salt out of the body; for the greater the urine volume, the larger the fraction of the filtered chlorides which escapes reabsorption.

The decrease in urinary chloride concentration after cortico-adrenal extract administration could possibly have been brought about in one of two ways: by increased reabsorption of chlorides, or by decreased reabsorption of water (see fig. 2, Britton, Silvette and Kline, 1938). When reduction in urea concentration per cubic centimeter of urine, which occurs simultaneously with the reduction in chlorides, is taken into account, however, one is led to the conclusion that the effect of cortico-adrenal extract on the kidneys is mainly to decrease reabsorption of water by the tubules. The

hypothesis is now advanced that in the excretion of water and sodium chloride by the kidney, the hormone of the adrenal cortex acts antagonistically to the antidiuretic factor of the posterior lobe of the pituitary (fig. 1). Normal secretion of both hormones would thus be necessary for the maintenance of sodium chloride and water balance in the body.

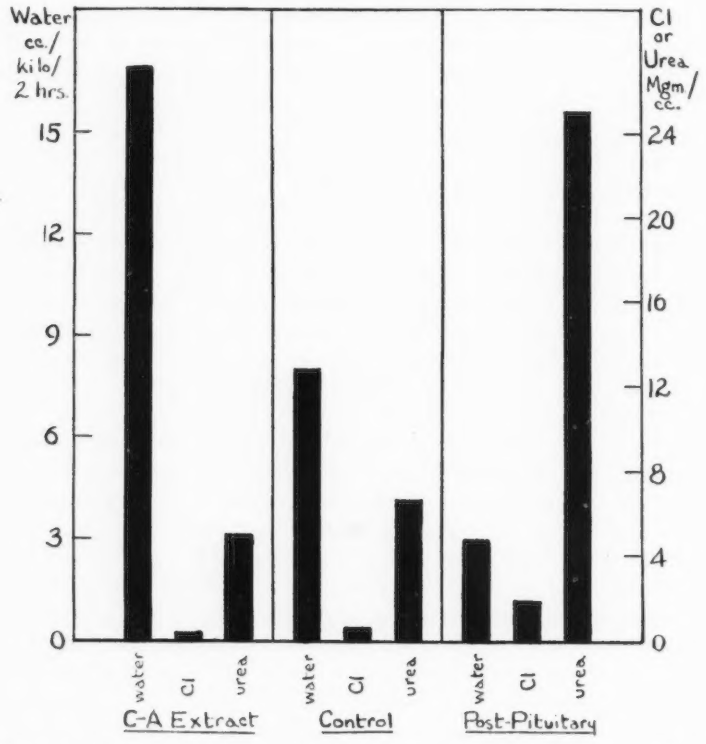

Fig. 1. Graph showing physiological antagonism between cortico-adrenal extract and post-pituitary solution in adrenalectomized opossums receiving standard quantities of water by mouth. Water, chlorides and urea excreted after treatment with cortico-adrenal extract or post-pituitary solution, and comparison with untreated controls.

The observation that the cortico-adrenal hormone is diuretic, and thus antagonistic to the antidiuretic hormone of the post-pituitary lobe, explains the hitherto puzzling fact that completely hypophysectomized animals show only a transient polyuria, while animals from which the posterior lobe alone has been removed show persistent diuresis. When the posterior lobe alone is removed, lack of its antidiuretic hormone plus

the presence of the diuretic adrenal hormone leads to polyuria because of decreased water reabsorption from the renal tubules. When both lobes are removed, the uncompensated activity of the diuretic cortico-adrenal hormone would persist only until the adrenal cortex (in the absence of the corticoadrenotropic hormone of the anterior lobe) becomes atrophic. At this time, then, both posterior-lobe antidiuretic hormone and cortico-adrenal diuretic hormone would be absent from the blood-stream, a sort of intrinsic balance would be restored, and polyuria would disappear.

In connection with the above, Winter, Gross and Ingram (1938) have observed no consistent or marked decreases in the concentration of serum sodium and chlorides following adrenal removal from cats with diabetes insipidus. Results of a further study also showed that in similarly treated animals there occurred a decline in the polyuria (Ingram and Winter, 1938).

We believe that, although the main experimental data on which this theory of cortico-adrenal-post-pituitary antagonism is based have been derived from a study of the opossum, the hypothesis may explain the adreno-hypophyseal relationship in other animals as well. Thus, normal and adrenalectomized opossums react to injected salt and water in the same manner as does the white rat (Silvette and Britton, 1933, 1938). Serum sodium and chloride (and also carbohydrate) levels in long-surviving (2–4 weeks) adrenalectomized opossums are reduced to the characteristic low levels reported for other species (cat, dog, rat, etc.) (Britton and Silvette, 1937). In the present metabolic experiments the great majority of the opossums which were adrenalectomized and subsequently used pursued a slow decline until death from adrenal insufficiency occurred a few weeks (average 17 days) later; and serum analyses which were made of several such animals showed low sodium and chloride levels.

Moreover, many observations by ourselves and other workers on common laboratory animals support the conclusions drawn from a study of renal function in the opossum. Adrenalectomized rats on an *ad libitum* water intake excrete subnormal amounts of urine, greatly increased amounts of chlorides and normal quantities of urea (Silvette and Britton, 1933). A similar picture has generally been observed in normal animals injected with post-pituitary solution. We have also shown that both rats and cats suffer hydration of liver and muscle tissues following adrenal removal, and this again is corroborative of the present theory. Administration of cortico-adrenal extract to adrenalectomized dogs results, furthermore, in diuresis (Swingle, 1937) and retention of salt (Harrop, 1937); and a similar effect is observed after hypophysectomy.

The principle in our cortico-adrenal extracts which affects urinary secretion appears to fulfill all the requirements of a true diuretic substance as set forth by Smith (1937, p. 229).

The present hypothesis of the physiological antagonism between hormones of the adrenal cortex and posterior lobe of the pituitary seems, indeed, to answer many of the hitherto unanswered and inexplicable questions which have been posed by renal physiologists.

SUMMARY

Cortico-adrenal extract, administered to opossums routinely by mouth, was effective in producing changes in renal excretion in both normal and adrenalectomized animals. Intraperitoneal injection of the extract was, however, somewhat more effective.

Adrenalin injection was without significant effect on renal output of normal and adrenalectomized opossums. Post-pituitary solution (Squibb) produced the typical antidiuretic effect when injected into water-fed opposums, but antidiuresis did not occur when the urine was highly concentrated, i.e., after salt or salt-urea administration. Concentration of chlorides and urea per cubic centimeter of urine was increased after post-pituitary administration.

Cortico-adrenal extract administration to normal and adrenalectomized animals brought about increases in fluid excretion coupled with decreased elimination of chlorides and urea per cubic centimeter of urine. When the urine excreted was highly dilute, however, further diluting effect of the cortico-adrenal hormone was not in evidence.

On the basis of these and other considerations, herein discussed, the hypothesis is advanced that in the excretion of water and sodium chloride by the kidney, the diuretic hormone of the adrenal cortex acts in physiological antagonism to the antidiuretic hormone of the posterior lobe of the pituitary. Normal secretion of both hormones is thus necessary for the proper maintenance of sodium chloride and water balance in the body.

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ALUMINUM IN THE NUTRITION OF THE RAT[1]

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Received for publication May 18, 1938

The part played by aluminum in the nutrition and metabolism of experimental animals has attracted considerable attention in the last two decades. Since this interest was aroused by the use of aluminum utensils in cooking, by far the greater proportion of the research has been directed toward the possible toxic effects of salts of this metal (1, 2, 3, 4) rather than toward its possible essential rôle in animal physiology.

Aluminum is universally present in traces in animal and plant tissues. This fact has been confirmed by nearly all workers in this field with the exception of McCollum, Rask and Becker (2) who used the spectrographic method of analysis. They state, "Aluminum is not a constituent of either plant or animal matter." However in a later publication (3) they state, "We interpret our results as indicating the absence of aluminum down to less than 0.5 p.p.m. of the fresh tissue." Kahlenberg and Closs (5), however, using the same method, found aluminum present in appreciable traces in a wide variety of natural plant products.

Studies on the absorption of aluminum from the intestinal tract have, in general, shown little or no storage of this metal and a quantitative excretion in the feces when high levels were fed (6, 7, 8).

Myers and Mull (8) have investigated the effect of feeding small amounts of aluminum salts (2 mgm. of Al per day) on the growth rate and reproduction of rats. They conclude "the aluminum fed rats showed a slightly greater initial growth." There was no effect on reproduction in their rats through four generations. No attempt was made by them to free their basal ration from aluminum and no figure for its aluminum content was given. Daniels and Hutton (9) found that rats on an all milk diet supplemented with iron and aluminum salts showed perfect reproduction for three generations. Supplementing with iron alone allowed no reproduction. Since this work was done before the essential rôle of copper and manganese had been recognised its significance is not clear.

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

In view of these indications of the essential rôle of aluminum in the growth and reproduction of the rat we have reinvestigated the problem using mineralized whole milk as the basal ration.

EXPERIMENTAL. The method for aluminum estimation was essentially that of Eveleth and Myers (10) with two modifications: First, the iron was separated from the aluminum by dissolving the combined phosphate precipitate in *conc* HCl, adding one drop of 30 per cent hydrogen peroxide and extracting with *dry* ether in a continuous extractor. Second, the final color-lake was filtered onto a half inch $BaSO_4$ mat (12). This refinement greatly increased the sensitivity of the method, allowing estimation of aluminum to within 0.5 microgram with an optimum range of from 1 to 8 micrograms. A recovery of 90 to 100 per cent was obtained when 3 and 5 micrograms of aluminum were added to milk samples.

In the growth and reproduction studies rats were placed upon a whole milk diet supplemented with iron, copper and manganese salts, and different levels of aluminum salts. The milk used throughout the experiment was obtained from a single cow and collected directly into a glass container. The aluminum content of this milk on three different occasions was found to be 18, 21 and 27 micrograms per liter. Assuming a 50 cc. daily milk intake, each rat received 1 to 1.5 microgram aluminum per day from this source. The iron salt was prepared as follows: Pure iron wire was dissolved in HCl, *conc* NaOH added until the iron was precipitated as completely as possible. The precipitate was washed with the NaOH solution and redissolved in distilled HCl. Any aluminum contaminant would be removed in this process as the sodium aluminate. A "C.P." grade of the Cu and Mn salts was used. No aluminum could be found in a 4 cc. volume of this mineral mixture (1 cc. being the daily dose).

Stock female rats with two week old litters were placed in monel metal cages (screen bottom). Milk was fed ad libitum. The mothers were removed twice a day for two hour periods to stock feed cages. The litters were limited to six rats. On weaning, the young rats were placed in individual monel metal cages and divided into three groups; aluminum supplements were fed at the levels of 0, 10 and 30 micrograms per day as $Al_2(SO_4)_3$. Growth records were kept for six week periods. The results are summarized in table 1.

Three females and two males in each group were tested for ability to reproduce. All were fertile except a female in the control group and one in the group receiving 30 micrograms of aluminum per day. One male getting 10 micrograms aluminum per day was sterile. Estrous cycles of the females were normal. All litters were normal, varying from 4 to 8 in number and all alive. However, none of the young survived for more than four days. This high death rate can be explained, at least in part, by the fact that the young were born on screens and so probably died from

cold. Milk was present in the stomachs of all the young on the first day of life.

Aluminum analyses on the blood and liver of rats of the three groups at the end of the six week growth period were made. Composite samples were used. The results are shown in table 2. The blood was obtained from the aorta of the anesthetized rat with a 10 cc. hypodermic syringe.

DISCUSSION. The data presented in table 1 indicate that aluminum has a slight stimulatory effect on the growth of rats during the six week period following weaning. The growth difference is so slight however (0.3

TABLE 1

Average daily growth of rats on mineralized milk with aluminum supplements

(6 weeks' period from weaning)

GROUP	MALES			FEMALES		
	Number of rats	Growth		Number of rats	Growth	
		Range	Average		Range	Average
		grams per day	*grams per day*		*grams per day*	*grams per day*
Control	7	3.00-3.64	3.24	8	2.10-2.93	2.41
+ 10 micrograms aluminum per day	7	3.11-4.05	3.56	8	2.10-3.26	2.77
+ 30 micrograms aluminum per day	8	3.20-4.33	3.50	6	2.14-3.22	2.39

TABLE 2

Aluminum content of blood and livers of rats on mineralized milk with indicated aluminum supplements

GROUP	BLOOD (MICROGRAMS ALUMINUM PER 100 CC.)			LIVER (MICROGRAMS ALUMINUM PER KM. FRESH)		
	Composite sample from	♂	♀	Composite sample from	♂	♀
Control	3 rats	13.2	14.7	2 rats	90	107
+ 10 micrograms aluminum per day	3 rats	15.6	13.6	2 rats	130	140
+ 30 micrograms aluminum per day	3 rats	23.0	20.0	2 rats	265	290

gram per day average) and the number of animals so small that no positive conclusion is permissible. It is apparent that if aluminum is needed at all by the rat (in addition to the indicated slight growth response) the requirements are satisfied by the one gamma per day received in the milk. The observation of Sharpless (12) that aluminum can counteract the toxicity and growth inhibition due to fluorine has no bearing on the present problem since such small quantities are involved. The milk consumed by a rat per day (about 50 cc.) contains only from 5 to 8 micrograms of fluorine, which would hardly be sufficient to produce a growth inhibition.

Cow's milk has proven to be lower in aluminum than a highly purified synthetic ration used for zinc deficiency studies, which contains 220 micrograms of aluminum per kilo. Milk contains about 150 micrograms per kilo of dry matter. A stock ration contained 2500 micrograms per kilo.

It is evident from table 2 that aluminum is absorbed from the intestinal tract and stored in the liver, when small amounts are fed. The rats had been killed for analysis 6 hours after the last aluminum-supplement feeding. The low figure for the aluminum concentration of the blood of the group receiving 10 micrograms per day indicates a fairly rapid removal of absorbed aluminum from the blood stream. The 40 per cent and 300 per cent increase in the aluminum concentration of the livers of the supplemented rats is evidence that storage of the absorbed aluminum occurs. The negative results obtained by some workers on this problem can readily be explained by their use of macro- or spectrographic analytical methods of analysis, and by the feeding of high quantities of aluminum salts, which would tend to blot out the significance of small differences.

SUMMARY

1. Forty-five rats (mixed males and females) were divided into three groups, placed on a constant mineralized milk diet, and supplemented with 0, 10 and 30 micrograms of aluminum (as aluminum sulfate) per day.

2. During the six weeks period from weaning the rats receiving additional aluminum, with the exception of one group of females receiving 30 micrograms of aluminum daily, showed a slightly, but really insignificant, better growth (0.3 gram/day).

3. Reproduction and the estrous cycle were normal in all three groups.

4. Analyses of the blood and livers of rats from the three groups showed definite absorption and storage of aluminum.

5. It can be concluded that, if aluminum is needed in the nutrition of the rat during its most rapid period of growth, one microgram per day received in its milk will satisfy this requirement.

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THE INFLUENCE OF VASCULAR FACTORS ON MEAN PRESSURE, PULSE PRESSURE AND PHASIC PERIPHERAL FLOW

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Received for publication May 20, 1938

Phenomena which are seemingly obvious and simple often prove to be obscure and complex on further analysis. This applies to interpretations of the relative rôles that peripheral resistance and arterial elasticity play as determinants of pulse pressure, mean pressure and phasic variations in peripheral flow. For instance, Böger and Wezler (1) apparently voice the opinion of the Munich group of physiologists in their statement, "granting a constant minute volume it remains questionable whether arterial elasticity or total peripheral resistance chiefly determine mean blood pressure levels." Similarly, differences of opinion exist as to whether augmented peripheral resistance, sufficient to elevate pressures above normal ranges, decreases or increases pulse pressure amplitude. It has been accepted since the early studies of Marey (2) that pulse pressure decreases in equally distensible tubes as peripheral resistance increases. However, as O. Frank (3), Erlanger and Hooker (4) and others have pointed out, this tends to be counteracted at higher pressures—variously placed as starting at 80 to 140 mm. Hg—by the progressive decrease in extensibility of arterial walls, which operates to augment pulse pressure. What the resultant of these opposing factors may be, has been differently interpreted. On the basis of experiments on circulation models, first reported in 1919 (5), I concluded that in normal arteries the diminishing effect of augmented resistance continues up to fairly high pressures. This seemed to be confirmed by additional experiments on perfused aortae of dogs removed from the body (6). Hochrein (7) applying the mathematical analysis of Frank asserts that only an increased pulse pressure may be expected. Bazett (8) in tabulated fashion implies that increased resistance has no effect on pulse pressure.

Extensibility and volume elasticity curves of the aorta and large blood vessels have been repeatedly established on vessels obtained post mortem. We need refer only to the recent studies of human aortae by Hochrein (7), O. Frank (9), Hallock and Benson (10), Krafka (11) and others.

While it may be questioned whether an extensibility curve or modulus of elasticity of aortic strips and rings, or volume elasticity coefficients calculated from short cylinders of aorta, give accurate pictures of the volume-elasticity relations of the whole aorta, not to mention its branches, it may be provisionally accepted that such data indicate general trends.

Unfortunately, changes in elasticity corresponding to those incident to ageing or pathological processes cannot be reproduced in animals; indeed, were this possible, the effects would again be complicated by other changing coefficients. For this reason, physical tests were again made on a circulation model, not with the idea that facts so gathered are final or even necessarily applicable to living animals and man, but because they allow at least a first approximation toward the guiding principles which can then be further tested on living animals.

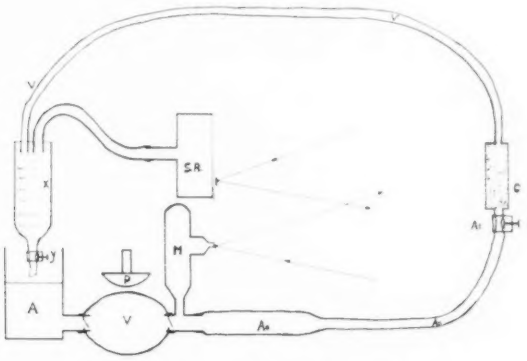

Fig. 1. Diagram of circulation model

Apparatus. The apparatus used was similar to that already described (12) and is schematically shown in figure 1. A rubber bulb, *V*, fills by suction from a glass container, *A*, and when compressed by a plunger, *P*, expels a controllable volume of water into the "aorta," *Ao*. The mode of filling of the bulb is obviously inconsequential in studies of arterial pressures.

The chief additions were 1, a calibrated optical manometer, *M*, peripheral to the "aortic" valve for recording arterial pressure pulses, and 2, a graduated flow chamber, *X*, connected to a large segment recorder, *SR*. The latter recorded *total flow* for a beat or two when the clamp, *Y*, was temporarily closed and *equilibrated flow* when clamp, *Y*, was adjusted so that inflow and outflow were equal. The equilibrated flow was particularly useful in compensating quickly for any change in peripheral flow due to vascular factors by changing the output of the bulb. The venous

flow showed a considerable lag due largely to the use of rather long tubing between the flow chamber, X, and recorder SR. This lag was easily determined by compressing the arteries and determining the time differences between the artificial rise of the pressure and increase in the flow curves, e.g., at X-X' in figure 7. In general, the capacity of the system was about 60 cc. and the systolic discharge, about 10 to 12 cc.

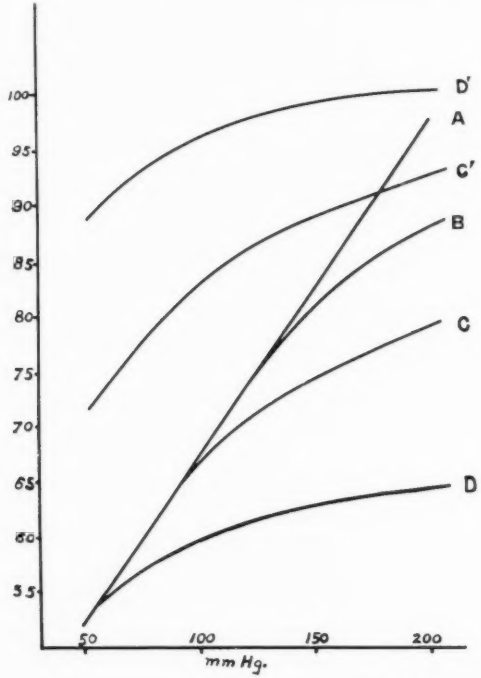

Fig. 2. Volume elasticity curves attained by placing rigid tube over Ao, figure 1, so that expansion was prevented beyond a fixed internal pressure. Description in text.

Peripheral resistance was created by tightening a micrometer screw, At. This proved to be more natural than the widely adopted expedient of the Starling heart-lung preparation which collapses during each diastole and permits a flow into capillaries during systole only. The large artery, Ao, could be wholly or partly surrounded by two halves of a metal casing, hinged on one side. In this way expansion of this tube could be limited at any desired internal pressure. Since the remainder of the tubes retained their normal elasticity, rapidly decreasing distention of the whole system could be produced at different pressure levels.

In order to study effects of capacity, the respective tubes could be lengthened or replaced by larger ones of the same material. However, since the effects on pulse pressure depend upon the ratio, systolic discharge: capacity, identical effects could be more easily obtained by keeping the tubes of the same diameter but decreasing the systolic discharge by the correct amount.

The relation of pressure pulses and pulse pressures to changing volume-elasticity relations. The effects of sequential increases in peripheral resistance were studied under a great variety of elastic conditions of blood vessels. This report is limited to the conditions indicated in the curves of figure 2.

Curve *A* represents an ideally expanding system in which the volume increment is the same for each additional pressure increment. In curve *B*, the distensibility is likewise proportional to pressures up to 110 120 mm. Hg, but beyond this range, decreases somewhat. Such a curve is fairly representative of conditions in early adult life. In curve *C*, the diminishing distensibility $\left(\text{increasing } \frac{dp}{dv}\right)$ begins at lower levels, *circa*, 80 to 90 mm. Such curves approximate those obtained from human aortae during middle adult life. In curve *D*, expansion is much reduced at very low pressures and the total expansion is never great. It resembles those of old age and of younger individuals with arteriosclerosis. Curves *C'* and *D'* illustrate the changes that occur when $\frac{dp}{dv}$ remains the same as in *C* and *D* respectively, but the volumes of the system at 20 mm. Hg internal pressure are respectively 35 and 70 per cent greater with respect to systolic discharge.

The effects of gradually increasing the resistance under these different conditions are represented by correspondingly labelled records of figure 3. When distensibility is equal for increasing pressures the effects of increasing peripheral resistance are those well known. Each increase decreases the slope of the diastolic limb, raises diastolic more than systolic pressure and thereby reduces the pulse pressure amplitude. The curve so nearly resembles that of figure 3-B that it is omitted to conserve space.

Record B. The pressure amplitude diminishes and the diastolic gradient decreases with increasing peripheral resistance, as in tubes with perfect distensibility. The diminishing distensibility of tubes above 120 mm. Hg is qualitatively without effect on the pressure curves. The only difference is a quantitative one; the decrease in amplitude is not quite as great. Similar effects are obtainable with greatly varying ratios of systolic discharge: arterial capacity. The conclusion seems reasonable that moderate changes in $\frac{dp}{dv}$ at pressures above 120 do not alter the fundamental di-

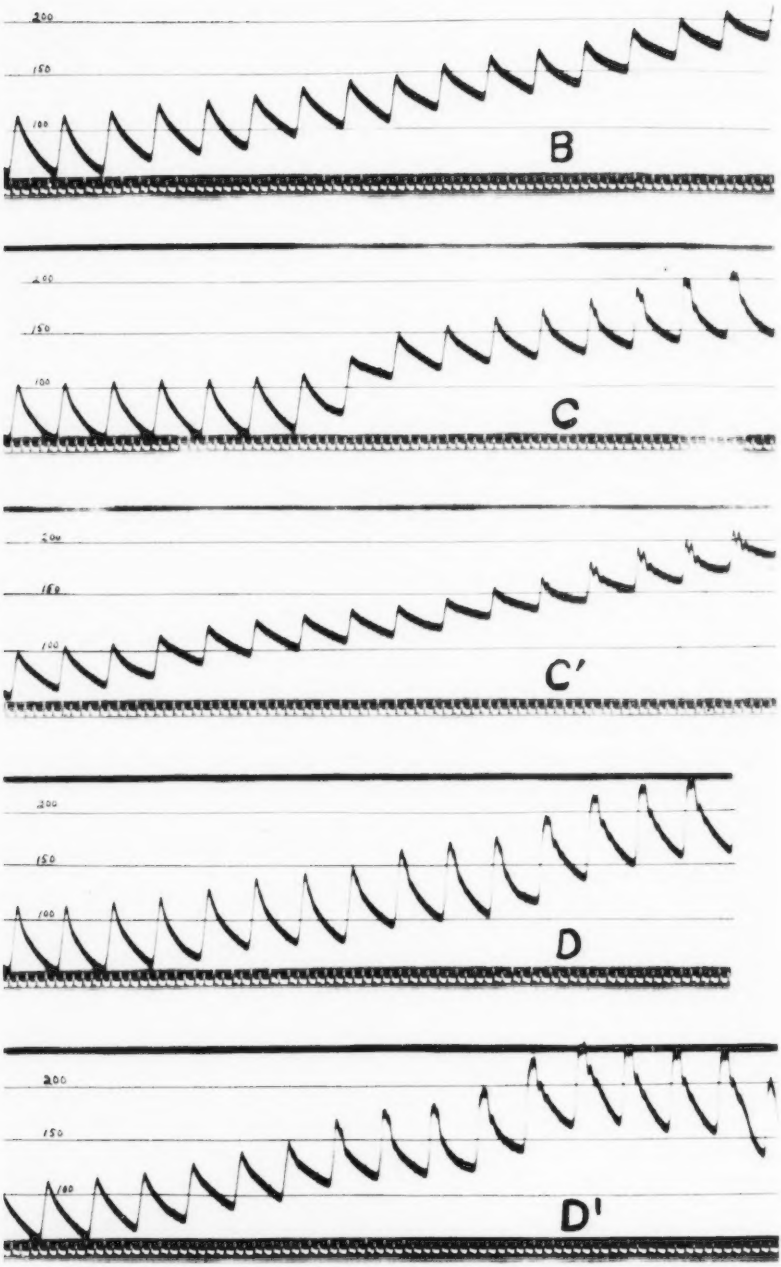

Fig. 3. Optical pressure pulses obtained during continuing increase in peripheral resistance in systems with volume-elasticity curves lettered similarly in figure 2.

minishing effects of increased peripheral resistance on pulse pressure amplitude.

Record C. The primary effect of a rather abrupt increase in peripheral resistance is to decrease pulse pressure and retard diastolic decline of pressure. As resistance increases more, the effect of diminishing expansion for equal pressure increments first causes changes in contour. The diastolic gradient becomes steeper, the summits are peaked and the amplitude tends to increase slightly. All of these changes happen before the pulse pressure again equals that existing at the beginning of the record pressures. Not until the beat indicated by the arrow, is pulse pressure greater than at the start. Obviously statements as to the effects of peripheral resistance on pulse pressure amplitude in such a system need to be qualified, e.g., the pulse pressure decreases when diastolic pressure is raised from 60 to 140 mm. Hg, whereas it increases when it is elevated from 110 to 140 mm. Hg or over. No general statement and no mathematical formula can express the anticipated changes in amplitude; because the factor denoting the relationship between increasing capacity and diminishing distensibility is not constant as internal pressure rises.

Record C'. The successive changes in form and the tendency of amplitude first to decrease and then to increase are similar when the capacity of the system with respect to cardiac output is increased 35 per cent. However, the pulse pressure amplitude never exceeds that at the start of the record; the significant changes occur in the form of the pressure pulse.

Record D. The effects are those well known to occur in comparatively rigid systems. The form of the pressure pulse alters and its amplitude increases with the first increase in peripheral resistance. Systolic pressure increases markedly and diastolic, much less, due to the rapid decline of pressure during diastole. There can be no question but that such a marked increase in $\frac{dp}{dv}$ completely reverses the effect that increased peripheral resistance has in more elastic tubes.

Record D'. The effects are similar but reduced when the capacity of the arterial system is increased in addition.

If the elasticity curves of aortae in animals and man studied after death portray at all accurately conditions existing during life and if we transfer to them physical phenomena obtained under similar states in elastic tubes, the conclusion must be drawn that increased peripheral resistance tends to diminish pulse pressure up to levels *circa* 200 mm. Hg, except when the modulus of elasticity is greatly increased, i.e., late in life or as a result of sclerotic changes due to disease. The importance of diminishing elasticity with increasing pressure as a factor determining directional changes in pulse pressure has been greatly over-estimated by those who choose theoretical considerations alone as a guide.

It should be stated that in records such as those reproduced in figure 3 the peripheral minute flow and hence the cardiac output, decreased somewhat. This reduction in systolic discharge actually made the pulse pressures somewhat smaller than in tests in which this was compensated. However, as shown in figure 4, this does not alter the directional trend.

The volume elasticity and pulse pressure relations of the dog's aorta in situ. In 1932 (6) I reported that a reduction of pulse pressure similar to that shown in figure 3-B occurs through increased peripheral resistance when a dog's aorta, carefully removed from the body immediately after death and with all branches ligated, is substituted in an artificial circuit. Since minor leaks were difficult to control, the pressures could not be elevated beyond 160 mm. and only crude volume elasticity curves could be constructed. Furthermore, the possibility exists that in the body the aorta, being bound down by surrounding adventitia, may be less expansile.

Experiments were therefore performed on dogs, recently dead, with their aortae in situ. To obviate ligation of numerous branches, a very thin "inner tube," 5 mm. in diameter,[1] was passed down the descending thoracic aorta to the division into the iliac arteries. This preparation (25–30 cm. long) was connected to the pump and optical manometer shown in figure 1 in place of the arterial system, *Ao* and *At*.

The typical records of figure 4 show, in addition to the volume elasticity curve determined at the close of the experiment, the same trend of diminishing pulse pressure with increasing peripheral resistance. In this instance, the peripheral outflow and cardiac output were kept equal as shown by the equilibrated flow record just above the time marker. The volume elasticity curve is typical of those generally found in dogs. I have just discovered that Hürthle (13) has reported a similar curve for the whole arterial tree. The diminishing extension with increasing pressures occurs only at pressures near 200 mm. Hg in 10 dogs examined. The record of figure 5 from another dog shows the sequential effects of increasing peripheral resistance when the slight reduction in cardiac output was not compensated. The same diminution of pulse pressure and form of curves is noted, except that at pressures near 200 mm. Hg a tendency to increase becomes apparent during beats denoted by an asterisk (*). This, together with the sharper upstroke, the peaked summit, and steeper diastolic gradient, suggests that the effects of diminishing elasticity are coming into play. Nevertheless, the pulse pressure, though increasing slightly, is still less than at the beginning of the record. Unfortunately, in this experiment volume elasticity relations were not determined above pressures of 185 mm. Hg.

Such experiments on six dogs fail to give evidence that diminishing

[1] Previous tests showed that the diameter of empty aortae of dogs 7 to 10 kilo average about 5 mm.

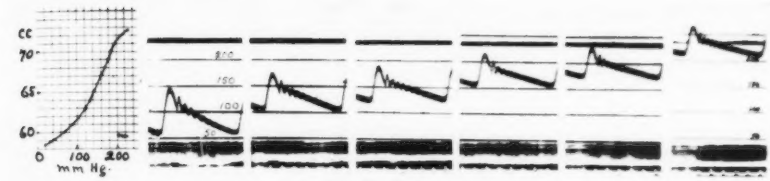

Fig. 4

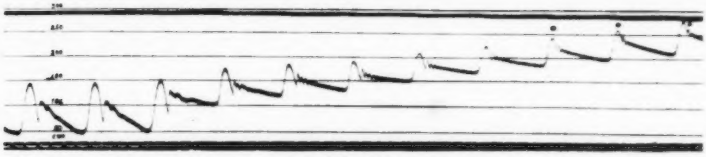

Fig. 5

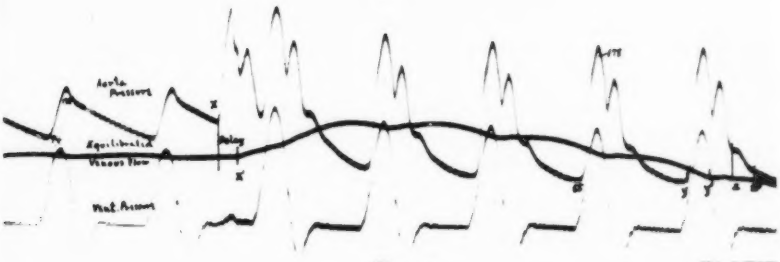

Fig. 7

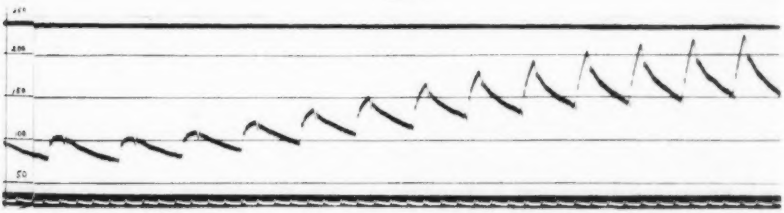

Fig. 8

Fig. 4. To left, volume elasticity curve of dog's aorta, in situ. Other curves, pressure pulses from same preparation during stepwise increase in peripheral resistance, the output and venous flow being restored to normal, as shown by white tambour shadow in black space above time signal.

Fig. 5. Pressure pulses from aorta of another dog during progressive increase in peripheral resistance. Note beginning effects of reducing distensibility in last three beats.

Fig. 7. Records showing immediate and stabilized effects of applying rigid casing to central artery of circulation model on aortic pressure (upper), ventricular pressure (lower) and equilibrated venous flow (middle).

At X, vessel compressed, thereby reducing its capacity and elevating arterial pressure artificially at X and after *lag* (X^1) it begins to show the first increase in flow. X-X^1; Y-Y^1; Z-Z^1; show corrections. Similar tests were made in all experiments.

Note diminishing equilibrated flow toward end of record, also that flow becomes pulsatile, owing to reduction of flow late in diastole (i.e., before Y^1).

Fig. 8. Effect of exciting central vagus nerve on aortic pressure-pulse of dog. Note increase in amplitude, steeper ascending plateau after initial steep rise of each curve and tendency toward a peaked summit and steeper diastolic decline in last 5 beats. Discussion in text.

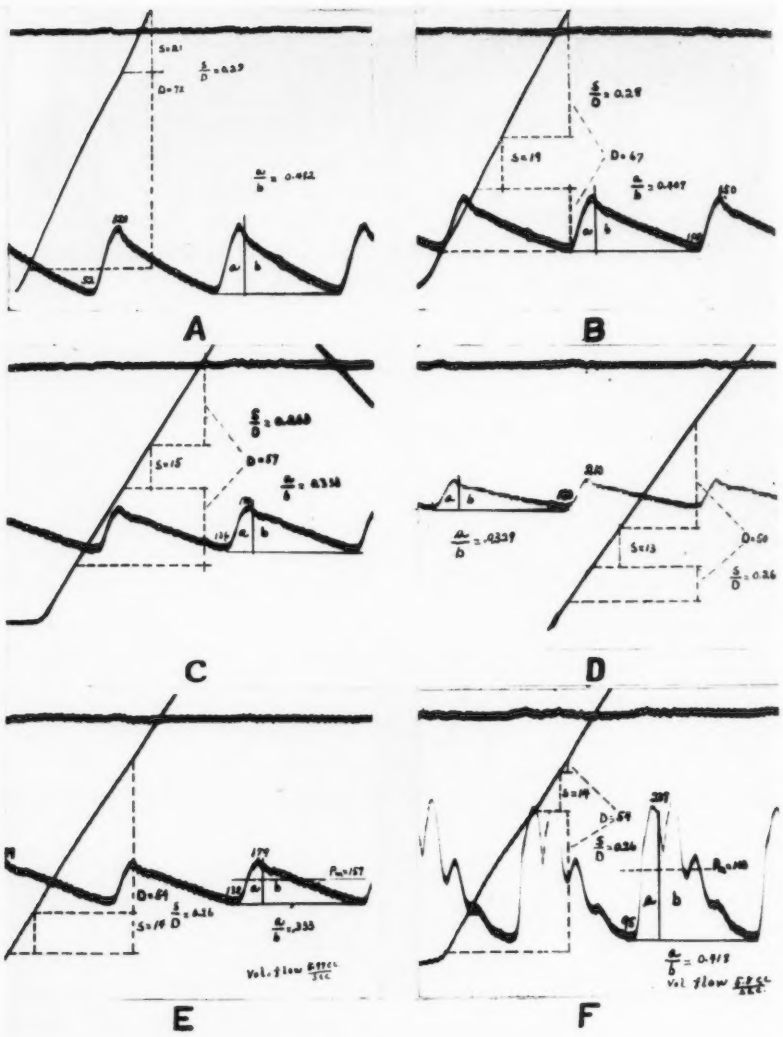

Fig. 6. *A*, *B*, *C*, *D*—pressure pulses and total venous flow during stepwise increase in peripheral resistance. Note decreasing pulse pressures, ratios, $\frac{a}{b}$ and $\frac{s}{d}$. *E*, *F*, effects of increasing $\frac{dp}{dv}$ of system. *F*, same peripheral resistance as in *E*. For correction of relative points see figure 7. Discussion in text.

elasticity of the intact aorta of dead dogs comes into operation, at least until pressures approach 200 mm. Hg.

The causes of reduced pulse pressure with increasing resistance. Two possible causes must be considered, viz., 1, a redistribution of peripheral outflow so that systolic flow becomes relatively greater or/and, 2, a reduction in the ratio, systolic discharge: arterial capacity.

Two approaches are available for determining whether a redistribution of systolic and diastolic peripheral flow occurs:

1. The mean pressures determined during systole and diastole, respectively, by integration of the systolic and diastolic portions of calibrated pressure curves seemingly represent the average pressures driving blood *from* the arterial system during these phases of the cardiac cycle. When the durations of systole and diastole are constant, a comparison of areas beneath systolic and diastolic portions of such curves accomplishes the same purpose. If the ratio of areas, $\frac{a}{b}$, figure 6, or their mean pressures are thus calculated, it is found that it decreases thus: record A, 0.492; record B, 0.407; record C, 0.333; record D, 0.329. In other words, systolic flow apparently decreases approximately from 49 per cent to 33 per cent of the diastolic flow in this system.

2. The venous flow—equal to the efflux from the arterial tree—during systole and diastole can be calculated directly from the vertical rises of the oblique flow curves, i.e., S and D. In the four curves, *A*, *B*, *C*, *D*, the ratio, $\frac{\text{systolic efflux}}{\text{diastolic efflux}}$, diminishes as $0.29 > 0.28$ and $0.263 > 0.26$.

Both criteria check in showing that the systolic run-off is relatively less, *not more*, as vasoconstriction increases and the total peripheral flow decreases. Therefore, the author's previous conclusion (12) that a greater systolic efflux contributes to the reduced pressure proves untenable. The pulse pressure decreases despite a slight shift in phasic flow toward diastole, by virtue of a reduction in the ratio, systolic discharge: arterial capacity. This is solely due to the increased capacity of the arterial system when cardiac output is restored to normal (fig. 4) and accentuated by the decreased systolic discharge, as in the curves of figures 3 and 6.

Calculation of relative systolic flows by these two methods obviously gives different answers, e.g., in the curves, *A*, *B*, *C*, *D* of figure 6, ratios such as $\frac{0.492}{0.29}, \frac{0.407}{0.28}, \frac{0.333}{0.263}, \frac{0.329}{0.26}$. The reduction is not even proportional. Such discrepancies can be explained if it be admitted that mean pressures calculated for periods of systole and diastole represent the forces driving blood from the central into the more peripheral distributing branches, but not necessarily out of the latter, through arterioles, into the capillaries. This could happen if, as Hürthle (14) suggested, the dis-

tributing arteries act as peripheral compression chambers which tend to buffer fluctuations in capillary flow. In the body, conditions are more complicated, for such accommodation of blood occurs earlier in branches of the aortic arch than in those of the coeliac axis, the renal and iliac vessels, etc. A time-distance factor enters causing a summation of the actions of different peripheral compression chambers.

Data presented and considerations arising from them make it improbable that total efflux from the arterioles during systole and diastole can be gauged by calculations of mean systolic and diastolic pressures. Further, any theory of hemodynamics which fails to take cognizance of the temporal summation of peripheral compression chamber actions does not offer a complete analysis of circulatory dynamics. These considerations are pertinent in attempts to calculate cardiac output from various physical measurements.

The cause of large pulse pressures with diminished aortic extensibility. The record of figure 6-E was obtained when the peripheral resistance and output had been readjusted approximately to the state represented in record C. Without any other change, the rigid casing was applied. The slight reduction in output volume accompanying this was compensated as indicated by the flow curves crossing the illustrations, E and F. The effects on the pressure pulses are typical. The curves rise and fall more steeply, diastolic pressure declines, systolic pressure rises and the pulse pressure becomes much larger. Integration of such curves demonstrates that mean pressure does not alter significantly, the slight directional changes, if any, being unpredictable. In curves *E* and *F* of figure 6 it rose from 157 to 163 mm. Hg. Similar observations were made by Fahr, Davis, *et al.* (15) on systems in which the heart itself was used as a pump. Observations such as these would seem to settle the question still considered unsolved by German investigators (1), whether changes in peripheral resistance or variations in arterial elasticity occurring concurrently dominate changes in mean pressure. Arterial elasticity certainly affects systolic and diastolic pressures, but no experimental evidence exists that it can alter mean pressure significantly. This is important, since physiologists accustomed to analyze mean blood pressure records as a resultant of central output and peripheral resistance changes may continue to do so in the old fashioned way. I see no reason for rewriting the hemodynamic principles as suggested in current German publications.

The extreme elevation of systolic pressure illustrated in figure 6-F is caused by the failure of aortic capacity to increase with respect to the volume ejected during systole; in other words a temporary (systolic) disproportion occurs between systolic discharge and aortic capacity. Since, however, less blood has been stored in the less distensible aorta, the diastolic outflow is much reduced, and the pressure sinks to a low diastolic

level. That this leads to an increased systolic and decreased diastolic flow from the aorta is demonstrated by calculation of the ratios, mean systolic pressure: mean diastolic pressure, or area *a*: area *b*. In these curves systolic flow increases from 33.3 per cent to 41.8 per cent of the diastolic.

This does not necessarily imply a similar increase in systolic flow through the capillaries. On the contrary, an analysis of the venous flow in these cases indicates that the systolic: diastolic ratio remains constant at 0.26. In instances in which peripheral resistance was not so great, systolic flow increased slightly in relation to diastolic. Such constancy must be attributed to the peripheral elastic reservoirs. These observations support the view that while comparison of mean systolic with mean diastolic pressures may offer an index of the phasic translocation of fluid from the aorta into its distributing branches, it cannot be considered an index of phasic efflux from arterioles of the arterial tree unless the distributing branches participate equally with the aorta in the decrease in elasticity.

Capillary and venous pulsations. An inspection of the "total flow" curves exhibited in figure 6-E and F demonstrates that although the total flow and the ratio, systolic flow: diastolic flow remains the same, the venous flow is no longer constant. The pulsatile character of the flow is shown even better in figure 7, in which the equilibrated flow is recorded. In short, a larger aortic pulse pressure may cause capillary pulsations without affecting the total flow or the phasic distribution and, it may be emphasized, despite the occurrence of considerable peripheral resistance. The idea that capillary pulsations can result only from vasodilatation is a physiological anachronism. These observations have a bearing upon the interpretation of capillary pulsations in aortic insufficiency, arteriovenous aneurysms and certain types of hypertension.

Careful scrutiny of the records makes it evident that such pulsations differ from those due to vasodilatation in that they do *not* show an augmented flow during systole, e.g., after y', figure 7. The capillary pulsations associated with a large pulse pressure are occasioned by a periodic reduction in flow late in diastole, e.g., before y', figure 7. In a physical sense, this implies that while the peripheral elastic vessels are capable of buffering the large increase in pressure during systole, they are incapable of supplying the force to drive blood through arterioles late in diastole when aortic pressure becomes very low.

The effect of vasomotor actions on aortic pressure pulses of dogs. When aortic pressures of anesthetized dogs are recorded by calibrated optical manometers and the peripheral resistance is decreased—as in sudden hemorrhage, through action of nitrites, etc.—the pulse pressure increases as expected from the analysis of physical experiments. If, on the con-

trary, peripheral resistance is increased either by direct stimulation of the splanchnic nerves or reflexly through afferent pressor fibers (central vagus, sciatic, sinus caroticus), contrary to expectations, and contrary also to observations of Hürthle (13) on smaller animals, the pulse pressure does not decrease; it invariably increases. The magnitude of the increase does not exceed 25 to 35 per cent when the heart rate is rapid, but may reach 200 per cent of the original when it is slow. A typical curve following central vagus stimulation is reproduced in figure 8.

Such effects could be interpreted to mean that the augmenting effect of diminishing elasticity on pulse pressure occurs at much lower levels either because the coefficient of elasticity is different during life or because pressor reflexes alter not merely the size of peripheral arterioles but also the capacity and elasticity of central elastic vessels. If this were the case, the general statement would be justified that in animals and man an increase in peripheral resistance causes larger pulse pressures in contrast to rubber tubes or dead aortae inserted in artificial models, in which the dominant change is a contrary one. Since records of ventricular volume curves in similar experiments invariably show that the systolic discharge is increased, the larger pulse pressure is probably due to this factor chiefly. Such augmented output may result from *a*, mechanical reactions due to some systolic retention (Wiggers and Katz, 16); *b*, increased liberation of epinephrine (Heymans *et al.*, 17), though this is questionable since the heart rate is not affected, or *c*, through rather sudden increase in venous return due to marked contraction of the spleen.

While a diminishing expansion or capacity of the large vessels with rising pressures cannot be entirely excluded in such experiments, the contour of the curves (fig. 8) is not such as to suggest such an action, at least until diastolic pressure has exceeded 150 mm. Hg and systolic pressure, 200 mm. Hg. Even these changes may be due to a relatively larger discharge during the earlier portion of ejection.

Apparently the observations on anesthetized dogs that direct or reflex vasoconstriction is attended by an increase in pulse pressure cannot be accepted as evidence that diminishing elasticity of arteries with rising pressure is a factor in augmenting pulse pressure, certainly until very high pressures are reached. This possibility, however, merits further study.

SUMMARY AND CONCLUSIONS

In view of contrary opinions as to whether changes in peripheral resistance or concurrent alterations in the modulus of elasticity with rising pressures dominate changes in mean pressure and pulse pressure, more physical tests were made on a circulation model. In this way, tubes with volume elasticity coefficients resembling those determined post mortem

in man could be employed, and the variations exhibited by optically recorded pressure pulses could be analyzed.

It was found that the diminishing effect that augmented resistance has on pulse pressure in tubes with linear volume elasticity relations persists until the distensibility decreases to a degree found only late in life or during pronounced arteriosclerosis. In such instances, pulse pressure augments decidedly. With lesser degree of rigidity the curves show characteristic changes in form, and while pulse pressure shows a tendency to become larger than in perfectly elastic tubes it is rarely greater at very high levels than at low levels of pressure. The conclusion is reached that the importance of diminishing elasticity in early and middle adult life as a factor determining the directional change in pulse pressure with increasing peripheral resistance has been over-emphasized by those guided chiefly by theoretical considerations.

No evidence could be obtained that diminishing elasticity with increasing pressure plays a dominant rôle in the dog's aorta in situ when it was perfused with pulsating pressures and the peripheral resistance was increased.

The chief factor determining the reduction in pulse pressure seems to be the progressive increase in capacity and reduction of the ratio, systolic discharge: aortic capacity. As long as the capacity continues to increase to a reasonable extent with rising pressures, the pulse pressure continues to decrease.

When the expansion of the aorta at any pressure is reduced below a minimum the effects of diminished distensibility exhibit themselves. They consist in a marked elevation of systolic pressure, a reduction of diastolic pressure and an increase in pulse pressure. It was found, confirmatory of observations by Fahr, Davis *et al.* (15) that the mean pressure is not affected significantly. Variations in the level of mean pressure in animal experiments may therefore be analyzed, as in the past, viz., as a resultant of cardiac output and peripheral resistance.

Integration of pressure pulses suggests that with lessened arterial extensibility the efflux from the aorta is increased considerably during systole and reduced greatly during diastole. Analysis of recorded variations of venous flow indicates, however, that unless the peripheral distributing vessels share in the reduction of elasticity they continue to buffer the flow through arterioles into capillaries with the result that the relative systolic and diastolic flows remain approximately the same. However, the buffering action is unable to maintain flow during the latter portion of diastole, hence capillary and venous flows become pulsatile. It is stressed that such pulsations may occur despite marked constriction of arterioles and that it differs from the capillary and venous pulses due to vasodilatation in that

the former are due to diminishing flow late in diastole, the latter to augmented flow during systole.

When pressure pulses are recorded from anesthetized dogs while peripheral resistance is increased by stimulation of pressor fibers, the pulse pressure does not decrease, it increases. In view of the observations that such stimulation is accompanied by an immediate and progressive increase in cardiac output, such experiments do not prove that the augmenting effect of diminishing elasticity is more pronounced during life or that it is brought into action by such stimulations. However, such a possibility deserves additional study.

In conclusion I wish to acknowledge the assistance of Dr. René Wegria, Miss Janet Dingle and Messrs. G. T. Kent and L. L. Williams in the performance of experiments which are the subject of this report.

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A STUDY OF THE CIRCULATORY FAILURE OF ADRENAL INSUFFICIENCY AND ANALOGOUS SHOCK-LIKE CONDITIONS

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Received for publication May 23, 1938

Recent observations indicate that the life-maintaining hormone of the adrenal cortex exerts a direct pressor effect upon the circulation in animals prostrate from adrenal insufficiency, and in adrenalectomized dogs which have had the arterial pressure reduced to shock levels by various procedures. This pressor action of the hormone is separable and distinct from either fluid or electrolyte changes which may occur (Swingle, Parkins, Taylor and Hays, 1937a; Parkins, Swingle, Taylor and Hays, 1938). The data presented in this communication also show that neither electrolyte nor internal fluid changes *per se* are adequate to account for the symptoms and death of the animal from adrenal insufficiency.

METHODS. The animals employed were healthy, vigorous, dogs which had been adrenalectomized 10 months to 2 years previous to use in the experiments, and maintained in normal condition by adequate daily injections of cortical hormone. At the time of use the dogs were at peak weight, eating full rations and revealed no deviations from normal in their blood and urine chemistry, fluid balance and arterial pressure. The daily maintenance dose of cortical hormone was omitted on the day the experiments started.

Appropriate references to the various methods used in the blood and urine studies have been frequently mentioned in previous papers of this series (Swingle, Parkins, Taylor and Hays, 1937b) and will be omitted here.

Experiment A. Effect of hypertonic saline injected intravenously upon the circulation of the dog prostrate from adrenal insufficiency. Five adrenalectomized dogs were used. The animals were placed in metabolism cages and the daily injections of hormone discontinued. They were permitted to develop severe symptons of insufficiency, and intravenously injected with hypertonic saline when they were so weak they could no longer stand or walk about. Table 1 shows the pertinent data obtained from study of

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a representative case. Since the remaining 4 animals of the series behaved similarly this case will be discussed in detail.

On 2/7/38 the animal was active, vigorous and enjoying normal health. The blood and urine chemistry were normal in all respects with the exception of the low hematocrit which is typical of adrenalectomized dogs maintained for long periods on cortical hormone. Extract injections were discontinued on this date. Five days later the animal was in collapse with low arterial pressure, low serum Na and Cl, increased serum K and marked hemoconcentration. At 2:30 p.m., 30 cc. of a 20 per cent NaCl

TABLE 1

Effect of hypertonic saline, cortical hormone, and water upon the circulatory collapse of adrenal insufficiency

DATE	TIME	BLOOD PRESSURE	PULSE PER MINUTE	SERUM SODIUM	SERUM CHLORIDE	SERUM POTASSIUM	HEMOGLOBIN	RED CELL VOLUME	R.B.C.	WEIGHT	WATER INTAKE	URINE VOLUME	URINE SODIUM	URINE CHLORIDE	URINE POTASSIUM	REMARKS
1938		*mm. Hg*		*m-eq per l.*	*m-eq per l.*	*m-eq per l.*	*gm. per cent*	*per cent*	*millions*	*kgm.*	*cc.*	*cc.*	*m-eq*	*m-eq*	*m-eq*	
2/ 7	10:45 a.m.	100	60	140.4	112.8	6.5	12.2	38.9	5.88	13.2						Extract discontinued
2/12	2:20 p.m.	50	120	126.8	98.8	8.1	17.9	53.1	10.3	12.5						Collapse
2/12	2:30–50			(30 cc. of 20% saline intravenously)												
2/12	2:52 p.m.	98	76													Gross symptoms disappeared
2/12	3:20 p.m.	70	72				12.6	39.8	6.64							Listless and lethargic
2/12	10:50 p.m.	54	124	141.1	115.4	7.3	16.4	48.3	8.44	12.1	0	185	24.1	16.5	6.9	Collapse-hormone intrav.*
2/13	10:00 a.m.	66	132								0	200	21.4	22.2	13.2	Improvement
2/14	11:45 a.m.	72	128	146.3	116.8	5.0	15.5	42.8	7.24	11.6	0	195	23.8	23.6	13.3	Water 580 cc. + 280 cc.
2/14	8:30 p.m.	84	124	130.1	103.2	5.9	12.6	42.5	5.82		860					Active and vigorous
2/15	10:10 a.m.	96	88								90	145	9.8	9.9	3.6	Appears normal
2/16	9:45 a.m.	96	84	134.8	102.8	6.4	11.5	36.2	5.46	12.0	95	160	5.9	9.3	8.6	Normal—Salted food
2/17	10:00 a.m.										710	120	3.8	6.4	2.7	3 gm. NaCl supplement
2/18	10:10 a.m.	104	88	147.1	116.8	4.8	9.7	27.4	4.68	12.5	690	255	81.7	56.7	7.1	Expt. discontinued

* Extract—3 cc. per kgm. body weight intravenously; and 3 cc. per kgm. daily, given in divided doses until recovery was complete.

solution representing 102 m.-eq. of Na were injected intravenously by way of the jugular vein, at the rate of 1.5 cc. per minute. Within 22 minutes the arterial pressure had risen from 50 to 98 mm. Hg. Symptoms disappeared and the animal seemed much stronger and brighter. The hemoconcentration was reduced to normal showing that marked blood dilution had taken place.[2] However, within the ensuing 8 hours an interesting

[2] The blood diluting effect of hypertonic salt in normal and shocked animals is well known, and a voluminous literature exists covering the subject. A recent paper containing a review of older work and numerous references is that of Gilligan, Altschule and Volk: J. Clin. Investigation **17**: 7, 1938. We are concerned here solely with the effect of hypertonic saline upon the shock-like collapse of the circulation in adrenal insufficiency.

phenomenon occurred. The arterial pressure slowly but steadily declined to low levels, the blood reconcentrated and symptoms of shock became evident. By 10:50 p.m. the animal was again in collapse despite the fact that the serum Na, and Cl remained normal and the serum K was somewhat reduced. The clinical condition of the animal was such that cortical hormone in amounts representing 3 cc. per kgm. was given intravenously. No food or water was offered. The following morning the animal was greatly improved. On 2/14/38 blood samples were taken. The dog since receiving extract had eliminated a total of 580 cc. of urine (containing large amounts of Na, Cl and K). He was given an equivalent quantity of tap water and an additional 280 cc. *ad lib.* The effect of water ingestion became evident within a few hours. The arterial pressure rose to 84 mm. Hg, but the serum Na fell from 146.3 m.-eq./liter to 130./m.-eq./liter. The serum Cl declined from 116.8 m.-eq./liter to 103.2 m.-eq./ liter. The hemoconcentration largely disappeared. The animal appeared normal, and was active and vigorous. It is significant that the rise in blood pressure and decrease in hemoconcentration occurred at the time when the serum Na and Cl had fallen to levels characteristic of the dog prostrate from insufficiency.

On 2/15/38 the arterial pressure was 96 mm. Hg, or within 4 mm. of the normal. The following morning samples were taken; the dog was normal in every way except for the low serum Na and Cl. He was fed salted ration and the experiment discontinued.

An experiment was performed on one dog which differed from the one described in that the animal was not injected with hormone following saline and all water was withheld. The essential data are shown in figure 1.

Five days after cessation of extract injections, the animal was in collapse. The arterial pressure was very low, the blood was concentrated, but the extracellular electrolytes revealed surprisingly little change. The serum Na had decreased but 3.1 m.-eq./liter from the normal, and the serum Cl had fallen but 6.8 m.-eq./liter. The serum K had risen 1.4 m.-eq./liter.

Hypertonic saline (30 cc. of a 20 per cent solution) was injected at the rate of 2 cc. per minute which represents an increase of 0.5 cc. per minute over the injections given the previously described series. When 15 cc. had been given, the arterial pressure had risen from 46 to 106 mm. Hg with great improvement in symptoms and return of activity. The animal became restless on the table and the injection was stopped for 10 minutes. During this short interval the arterial pressure fell to 83 mm. Hg but following resumption of injection it rose to 110 mm. Hg. After completion of the saline infusion the animal was placed on the floor and allowed to run about for a few minutes. Gross symptoms had disappeared. He was returned to the metabolism cage and all food and water withheld.

As shown on the graph, during the next few hours, symptoms returned, the blood pressure fell to shock levels and the blood reconcentrated. The serum Na and Cl remained at unusually high levels and the serum K increased slightly. No urine was voided at any time during the experiment. The dog was not given hormone and died at the end of the 5th hour from the time of salt injection.

The high concentrations of extracellular electrolyte might be considered as a contributory cause of the final collapse of this animal. However, this is unlikely, since control experiments on intact dogs injected with

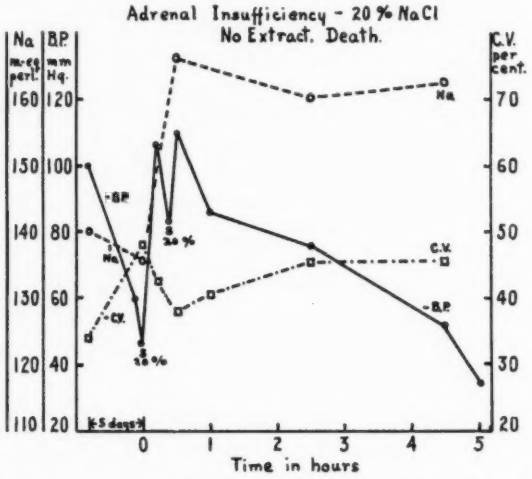

Fig. 1. Effect of intravenous injections of hypertonic saline (30 cc. of a 20 per cent solution) upon the circulatory collapse of adrenal insufficiency. *Bp* = arterial pressure in millimeters Hg; *Na* = serum sodium; *CV* = cell volume. The saline (15 cc.) was injected at points marked *S* on the graph with a 10 minute interval between injections.

similar quantities of saline, using the same rate of injection, show that these serum concentrations of Na and Cl are not deleterious during this time interval.

A rise in arterial pressure (averaging 18 mm. Hg in a series of 5 dogs) follows injections of 30 cc. of a 20 per cent solution of saline intravenously in the intact dog, but the increase is relatively small compared with the effect of similar injections upon the adrenalectomized dog exhibiting circulatory collapse. The pressure generally declines to normal within 30 minutes to 1 hour.

The profound and rapid effect of hypertonic saline upon the circulatory

collapse and hemoconcentration of the dog prostrate from adrenal insufficiency is due presumably, in large part at least, to the osmotic action of the salt in inducing blood dilution and thus temporarily transferring considerable volumes of water from extravascular spaces to the vascular system. Since the capillaries are freely permeable to crystalloids and fluid, the effect, however, is soon reduced by eventual establishment of osmotic equilibrium and hence dilution of the hypertonic solution with the mass of extracellular and intracellular water. Thus it might appear as though the circulatory collapse were primarily dependent upon loss of vascular fluid to the other body compartments and therefore the pressor action of cortical hormone was directly dependent upon the internal redistribution of body water. This, in fact, was our earlier view. However, if such were the case, all symptoms should disappear, and the arterial pressure remain high so long as the extracellular electrolytes are normal and the volume of circulating fluid is adequate. Both of these conditions are apparently fulfilled in these experiments yet the animals relapse into shock, reconcentrate their blood and exhibit circulatory failure within a few hours of salt administration (table 1 and fig. 1).

The evidence seems to indicate that the fault lies not with the extracellular electrolytes or amount of fluid available, but with the capillaries. The fluids returned to the blood stream by means of the hypertonic saline are not held in circulation. According to our interpretation, these animals can not hold their vascular fluids or dilute their blood in the face of the declining blood volume because the peripheral circulation becomes atonic following hormone withdrawal. The capillaries lose tone and dilate, thereby enormously expanding the periphery and creating a reservoir into which the vascular fluids drain and stagnate.

We have previously pointed out (Swingle, Pfiffner, Vars and Parkins, 1934) that although conditions at the vascular periphery of the adrenalectomized dog either in shock or adrenal insufficiency are extremely favorable for blood dilution and passage of fluid from tissues to blood stream, yet no such transfer takes place. The conditions favoring dilution are: low arterial pressure, low capillary pressure, slow blood flow and relative increase in plasma colloids. Yet dilution does not occur. We attribute the breakdown of the process of fluid exchange to capillary atony and the resulting dilatation, stasis and increased permeability. Following hormone injections the capillaries regain tone, the stasis disappears and free blood flow at the periphery is reëstablished.

That something is wrong at the periphery, and especially with the capillaries, is also shown by the following experiments which demonstrate that marked loss of vascular fluid, as indicated by the degree of hemoconcentration, is itself of little significance to an animal possessing adrenal

glands or to an adrenalectomized animal primed with cortical hormone, but is invariably fatal to the dog lacking hormone reserves.

Experiment B. Effect of hemoconcentration and loss of circulating fluid volume upon arterial pressure of intact, adrenalectomized primed with hormone, and non-primed adrenalectomized dogs on maintenance doses of hormone. The most striking hemoconcentration and loss of vascular fluids we have observed, occurs in the intact dog subjected to intraperitoneal glucose injections, representing 100 cc. of 5.5 per cent glucose per kilogram of body weight. Animals so treated recover and dilute the inspissated blood. The decrease in blood volume and increased hemoconcentration resulting from the glucose injections are of far greater magnitude than ever occur in adrenal insufficiency. However, the arterial pressure rarely

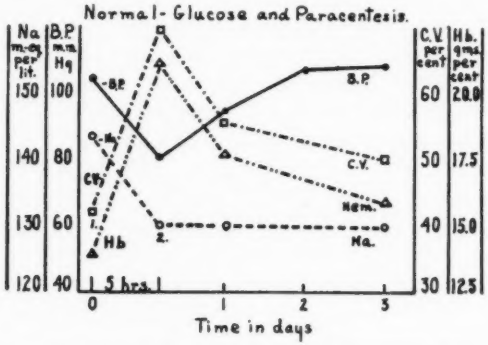

Fig. 2. Hemoconcentration in the intact dog resulting from intraperitoneal injection of isotonic glucose (100 cc./kgm.) followed by withdrawal of an equal quantity of ascitic fluid 5 hours later. *Bp* = arterial pressure in millimeters Hg; *CV* = cell volume; *Na* = serum sodium; *Hem.* = hemoglobin.

falls more than 20 to 25 mm. Hg in the intact dog (fig. 2). Study of many such animals has convinced us that the upset in electrolyte equilibrium and uptake of extracellular water by cells, (owing to excess renal elimination of sodium) which is a characteristic feature of adrenal insufficiency in the dog, is responsible for only a small part of the fall in blood pressure in the adrenalectomized dog exhibiting circulatory collapse from glucose injection or uncomplicated insufficiency. Therefore, one must look elsewhere to account for the circulatory failure in these conditions.

A comparative study was made of the reaction of the intact animal, the healthy, vigorous, adrenalectomized dog on maintenance doses of hormone, and the adrenalectomized dog primed with hormone, to intraperitoneal injections of 50 cc. per kgm. of 5.5 per cent glucose. Fifteen animals were studied. The results offer suggestive evidence that the

locus of action of the cortical hormone is upon the peripheral circulation and particularly the tonus of capillaries. The data are presented in figure 3.

As shown on the graph, glucose injections lower the arterial pressure of the intact and primed adrenalectomized dog approximately 20 to 25 mm. Hg, and recovery is spontaneous. On the other hand, the circulation of the non-primed animal collapses within 6 to 10 hours after 50 cc. per kgm. of glucose, and cortical hormone is required to restore the circulation.

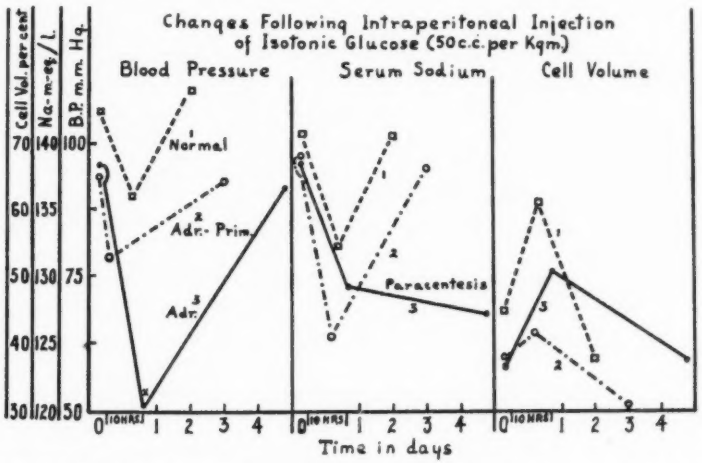

Fig. 3. Changes in blood pressure, serum sodium and cell volume following intraperitoneal injections of isotonic glucose (50 cc. per kgm.). *1 normal* = intact dog; *2 adr-prim* = adrenalectomized dog primed with large amounts hormone previous to injection; *3 adr.* = healthy, vigorous adrenalectomized dog on a maintenance dose hormone. Hormone was injected at point marked *X* on graph 10 hours after the glucose was given. *Paracentesis* = removal of acitic fluid 50 cc. per kgm. when animal was in shock. Ascitic fluid was not removed in animals 1 and 2.

The adrenalectomized animal primed with hormone just previous to glucose administration behaves like the intact dog to the injection. Symptoms do not appear, nor is there any evidence of circulatory disturbance aside from the initial temporary decline in arterial pressure which is soon spontaneously restored (fig. 3).

The probable explanation of the striking effect of glucose injections on the dog lacking hormone reserves is simply that such injections are merely another method of inducing circulatory shock in an animal which can not maintain the tonus of its capillaries when subjected to any sort of stress. The initiating factor in "glucose" shock is the small decline in arterial

pressure. Such a decrease is of no consequence to the intact or primed dog, but to the adrenalectomized animal lacking hormone reserves it becomes a very serious matter (fig. 3). The capillaries appear to lose tone when the animal is subjected to slight strain or stress and are unable to regain tone in the absence of adequate hormone. Circulatory collapse is a direct result of the peripheral atony and stagnation.

Discussion. Evidence is fast accumulating that the vital hormone of the adrenal cortex may exert regulatory control over several important body functions. For example, it apparently has some relation to carbohydrate metabolism (Britton and Silvette, 1932, 1932, 1935, 1937; Long and Lukens, 1936; Long, 1936–1937); and exercises some control of the renal excretion of certain electrolytes (Loeb et al., 1933; Loeb, 1935; Harrop et al., 1933; Hartman et al., 1938; Thorn et al., 1937, and others.) It is evidently somehow concerned with potassium metabolism (Kendall, 1937; Allers and Kendall, 1937; Zwemer and Truszkowski, 1937; Zwemer, 1937, Harrison and Darrow, 1938). According to Verzár and Laszt (1936) this hormone is also necessary for the phosphorylation of flavin. To this formidable list of regulatory activities of the life-maintaining hormone, should be added yet another function—control of capillary tone. This is a vital function since it is essential for normal fluid exchange between vascular and extra-vascular tissues, and maintenance of the circulation.

CONCLUSIONS

1. The pressor action of the adrenal cortical hormone upon the low blood pressure in dogs prostrate from adrenal insufficiency, is independent of changes in serum electrolytes, hemoconcentration or fluid distribution.

2. The adrenalectomized dog exhibiting circulatory collapse promptly reacts to intravenous injections of hypertonic saline by restoring the blood pressure and hemoconcentration to normal. The effect is temporary since the fluid is not held in circulation. Shock again develops and death from circulatory failure occurs unless adequate amounts of cortical hormone are administered.

3. The adrenalectomized dog (lacking hormone reserves) reacts to any procedure that throws a stress or strain upon the circulation (e.g., intraperitoneal glucose injection) by developing fatal circulatory shock, whereas similar animals primed with hormone and subjected to identical procedures react as does the dog with intact adrenals.

4. The writers attribute the cause of death from adrenal insufficiency to capillary atony, with resulting dilatation, stasis and peripheral vascular stagnation, and believe that the cortical hormone is concerned with the maintenance of capillary tone, and therefore regulatory control of the volume capacity of the circulatory system.

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EFFECT OF ADRENAL CORTICAL HORMONE UPON THE CIRCULATORY COLLAPSE OF ADRENALIN SHOCK

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Received for publication May 23, 1938

It is well known that massive doses of adrenalin produce fatal shock. This fact has been adequately demonstrated by Bainbridge and Trevan (1917); Erlanger and Gasser (1919); Trendelenburg (1924); Freeman (1933), and others. It was considered of interest to study adrenalin shock in the healthy, vigorous, adrenalectomized dog receiving maintenance doses of cortical extract and to determine whether or not the hormone was efficacious in reviving the animals from collapse.

The adrenalectomized animals were healthy and vigorous at the time of use. They had been bilaterally operated 6 to 18 months previously, and maintained in normal condition by adequate daily injections of cortical hormone. All food and hormone were withdrawn 18 hours previous to adrenalin administration. The dogs were table trained for blood pressure work and no anesthetic was used since the slow intravenous injections cause little if any pain or discomfort. Retching and vomiting occurred if the adrenalin was given too fast. When the animals were in profound shock with a falling blood pressure and it was evident that spontaneous recovery was unlikely, they were intravenously injected with adrenal cortical hormone in amounts representing 3 cc. per kgm. per day until recovery was complete.

Parke, Davis and Co. adrenalin hydrochloride was used. It was diluted with either 0.9 per cent saline or 5.5 per cent glucose and injected at the rate of 0.1 to 0.2 mgm. per minute through the saphenous vein. The rate was slowed when retching occurred, or occasionally the injection was stopped and the dog given a 10 minute rest period when symptoms of distress appeared. It is important that the adrenalin be given slowly.

The intact dogs used as controls were not trained for blood pressure work nor were they treated with cortical hormone when in shock.

The methods for blood and urine analysis, and arterial pressure determination, have been frequently referred to elsewhere in this series of papers (Swingle, Parkins, Taylor and Hays, 1937).

1. *Adrenalin shock in the intact dog.* Fourteen healthy mongrel dogs

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were used, of which 11 died in shock and 3 recovered spontaneously. The amounts of adrenalin used, expressed as milligrams per kilo of body weight, were as follows: 5 dogs received 0.66 mgm. per kgm.: 1 dog, 0.73 mgm.; 3 dogs, 0.8 mgm.; 5 dogs, 0.9 mgm./kgm. The total amount was injected as a 1–3000 solution. All of the animals developed profound shock, but three recovered spontaneously. Two of them received 0.66 mgm./kgm. and 1 received 0.8 mgm./kgm. Since but one animal survived a dose above 0.73 mgm./kgm. we assume that this is a close approximation to the minimum lethal dose of adrenalin necessary to produce fatal shock in the unanesthetized dog under the conditions of the experiment. Trendelenburg (1924) states that the lethal dose for the dog is from 0.2 to 0.8 mgm. per kilo. The administration of adrenalin apparently does not increase the rate of discharge of this substance from the medulla according to Sugawara, Saito and Nemoto, 1928; Tournade, 1926, and Rogoff, Marcus and Wasserman, 1938.

The symptoms of adrenalin shock are well known and need not concern us here. The arterial pressure attains high levels during injection but may decline abruptly shortly after completing the injection, or the fall may be slow but steady, extending over a period of hours. All animals dying from shock were autopsied. The heart was found to be dilated, the lungs showed congestion and were occasionally edematous and hemorrhagic. The gastro-intestinal tract was invariably hemorrhagic. The liver, spleen, pancreas, bladder and kidneys were congested and usually exhibited numerous small hemorrhagic areas. The adrenal cortex showed congestion and macroscopic hemorrhages were sometimes present. The intense congestion of the vascular periphery, and numerous hemorrhages, were the most striking gross changes encountered at autopsy of the shocked animals. Most investigators of adrenalin shock have reported similar autopsy findings.

Blood studies were made. The data obtained from two typical cases are presented in table 1.

Electrolyte and hemoconcentration changes in the intact unanesthetized dog injected with massive doses of adrenalin. Changes in serum Na, Cl and K were negligible, nor was there marked evidence of hemoconcentration even at the time of death. Dog A, table 1, revealed greater blood concentration 1 hour after injection than he did 3 hours later, as evidenced by the decline in hemoglobin, cell volume and R. B. C. two hours before death. Dog B exhibited some hemoconcentration which persisted until the end. The changes can not be regarded as significant.

Decline in blood volume and loss of circulating fluid have been reported for dogs and cats shocked with adrenalin (Lamson and Keith, 1915; Erlanger and Gasser, 1919; Freeman, 1933, and others). However, Gregersen (1936) states that in his experience, unanesthetized dogs injected

with large doses of adrenalin do not show consistent changes in blood volume. Some may show a decline in volume, others an increase, and still others remain unchanged. In so far as changes in blood concentration can be considered an index of volume changes, our data are in general agreement with Gregersen's statement. It is surprising, in view of the capillary dilatation and peripheral stasis present in the shocked dogs, that they do not exhibit more hemoconcentration than is evident. Erlanger

TABLE 1

Blood pressure, electrolyte and hemoconcentration changes in the intact unanesthetized dog injected with massive doses of adrenalin

DATE	TIME	BLOOD PRESSURE	PULSE PER MINUTE	SERUM SODIUM	SERUM CHLORIDE	SERUM POTASSIUM	HEMOGLOBIN	RED CELL VOLUME	R.B.C.	WEIGHT	DOSAGE PER KGM. WGT.	REMARKS
No. 6 ♂—A												
		mm. Hg		*m.eq. per l.*	*m.eq. per l.*	*m.eq. per l.*	*gm. per cent*	*per cent*	*millions*	*kgm.*	*kgm.*	
12/ 7/37	10:20 a.m.	112	88	143.4	110.8	4.3	15.1	48.0	8.0	18.1	0.9	Normal
	10:40 a.m.	150	140									Adr. inj. 10:20–11:20
	11:20 a.m.	120	136	139.8	108.4	4.1	17.5	56.8	10.2			Appears normal
	2:10 p.m.	54	200	144.9	112.8	3.9	16.3	53.0	8.21			Shock-Death at 4:15 p.m.
No. 8 ♂—B												
12/29/37	10:30 a.m.	110	92	142.2	110.4	4.6	13.2	39.4	5.89	12.5	0.9	Normal
	11:00 a.m.	134	128									Adr. inj. 10:30–11:45
	11:45 a.m.	116	120	143.0	109.8	4.1	14.6	44.1	7.36			Depressed inactive
	2:50 p.m.	48	224	146.4	113.8	5.8	14.9	44.7	7.48			Shock-Death at 3:00 p.m.

and Gasser (1919) believe that circulatory failure in adrenalin shock is due to the extreme slowing of the circulation throughout the body caused by the constricting action of adrenalin on the arteries. This eventually leads to tissue asphyxia, capillary dilatation, stasis, and sequestration of blood in the peripheral vessels and especially the capillaries and venules of the intestinal villi. Our experiments accord with this viewpoint, except that stasis and capillary dilatation are not confined to the gastro-

intestinal region but are apparently widespread throughout the body. Capillary atony appears to play an important rôle in this as well as other types of shock. Moon (1937) has stressed capillary atony as an important factor in shock of all types.

2. *The development of tolerance to massive doses of adrenalin.* Intact dogs develop a tolerance to sublethal doses of adrenalin. Vincent (1897) was apparently the first to call attention to this fact. He employed a crude extract of the glands and noted that "after a subfatal dose a partial immunity seems to be set up and it is difficult to cause a fatal result by a subsequent injection."

It was noted early in the present experiments that after the lapse of 10 to 12 days following recovery from adrenalin shock, the dogs reacted much less violently to larger doses than were originally used to induce shock. Continued use of these animals caused the development of such a high degree of tolerance that little effect was induced by administering quantities which are invariably fatal within a few hours to an animal not previously injected (fig. 1).

Since adrenalin is a pure hormone free from all contaminating material, the development of tolerance is of interest in relation to the theory of antihormones. Collip (1934) advanced the theory of antihormones to explain the observations that certain pituitary hormones when injected repeatedly will induce the formation of substances inhibiting their activity. The organism becomes refractory, and the particular end organ concerned no longer responds to the hormone even when it is administered in excessive amounts. According to the theory, every hormone is considered to have an antihormone, and the high concentration of antihormone induces the refractoriness. However, most investigators account for the refractory state by attributing it to an antigen-antibody reaction since purified hormones such as oestrone are said not to elicit the reaction (D'Amour, Dumont and Gustavson, 1934).

Four adrenalin-shocked dogs which had recovered from sublethal doses of adrenalin were studied over long periods and the essential data from a representative case are shown in figure 1.

This dog developed shock when intravenously injected with 0.66 mgm./kgm. of adrenalin, but recovered. Ten days later he received 0.9 mgm./kgm. which in our experience is a fatal dose. The animal did not exhibit any symptoms of shock and remained active. After the lapse of 10 days he was again injected, this time with 1 mgm./kgm. The pressor action of the hormone was not diminished but the dog did not show any effects, and at the end of the injection was as active and vigorous as before. Three hours following injection the blood pressure was normal and the animal ate a hearty meal.

Bauer, Kunewälder and Schachter (1937) report that administration

of pure thyroxin, di-iodotyrosine and adrenalin to rabbits in frequent doses over long periods results in a gradual loss of response. Sera from their injected rabbits gave a positive complement fixation test. They ascribed the positive results to the antigenic activity of the phenol nucleus in the molecules of the substances used.

3. *Adrenalin shock in the active, vigorous, adrenalectomized dog, and revival of the animal from collapse with cortical hormone.* Six animals were studied. They received varying amounts of adrenalin, ranging from 0.44 to 1 mgm./kgm. diluted with either 0.9 per cent saline or 5.5 per cent

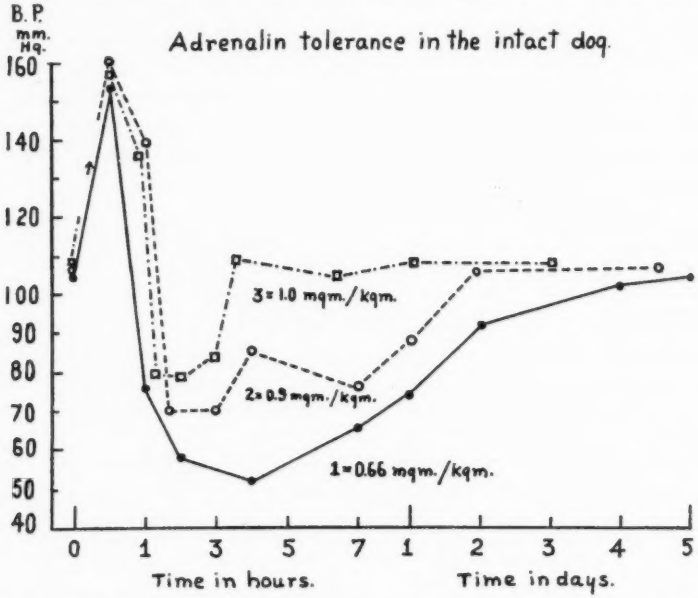

Fig. 1. The graph shows the development of tolerance in the intact dog to repeated large doses of adrenalin given 10 days apart. At 1 the dog was thrown into profound shock with 0.66 mgm./kgm. but recovered. Ten days later he was injected with 0.9 mgm./kgm. but failed to show symptoms. The third injection of 1.0 mgm./kgm. was without noticeable effect.

glucose. The adrenalin was given as a 1–5000 or 1–3000 solution and administered at a constant rate. Table 2 shows the pertinent data.

The serum electrolytes remain at normal levels in those experiments where the adrenalin was diluted with glucose, but show increases in Na and Cl when the injected material was diluted with saline. The serum K shows little change from the normal when the animal is in collapse. It is obvious that adrenalin shock has no relation to serum electrolyte changes. Using the hemoglobin, cell volume and R. B. C. as indices of

TABLE 2

Blood pressure, electrolyte and hemoconcentration changes in the active, vigorous adrenalectomized dog shocked by injecting massive doses of adrenalin†

DATE	TIME	BLOOD PRESSURE	PULSE PER MINUTE	SERUM SODIUM	SERUM CHLORIDE	SERUM POTASSIUM	HEMOGLOBIN	RED CELL VOLUME	R.B.C.	WEIGHT	DOSAGE PER KGM. WGT.	REMARKS
						No. 2 ♂						
	hours	*mm. Hg*		*m. eq. per l.*	*m. eq. per l.*	*m. eq. per l.*	*gm. per cent*	*per cent*	*millions*	*kgm.*	*mgm.*	
11/18/37	10:00 a.m.	110	84	146.1	115.8	6.8	12.8	34.5	5.42	13.6		Normal
11/18/37	11:45 a.m.	150	60								1.0	Adr. inj. 11:15–12:15
11/18/37	12:18 p.m.	64	128									Lethargic
11/18/37	2:00 p.m.	38	108	154.7	117.8	5.8	13.2	39.7	5.88			Shock, extract*
11/18/37	3:20 p.m.	55	136									Improvement
11/19/37	11:30 a.m.	72	108									H_2O given, no food
11/22/37	9:30 a.m.	104	92	140.1	103.2	5.5	12.8	34.7	4.50	12.3		Appears normal
						No. 3♂						
10/16/37	9:30 a.m.	110	80	140.3	111.2	3.9	8.4	27.3	4.08	9.5		Normal
10/16/37	9:52 a.m.	160	64								0.4	Adr. inj. 9:30–10:10
10/16/37	3:00 p.m.	90	212									Inactive
10/16/37	10:10 p.m.	54	212	143.3	117.8	3.1	8.9	28.5	4.29			Shock, extract*
10/17/37	9:40 a.m.	84	148									Much improved
10/18/37	1:30 p.m.	108	96	143.6	117.8	4.8	7.9	27.5	3.48	9.0		Normal
						No. 5 ♂						
11/17/37	10:00 a.m.	106	76	145.6	113.8	5.3	12.5	36.0	6.16	12.1		Normal
11/17/37	11:00 a.m.	156	68								0.66	Adr. inj. 10:00–11:20
11/17/37	11:55 p.m.	56	140	143.6	116.2	5.5	13.1	39.7	7.32			Shock, extract*
11/18/37	1:00 a.m.	68	148									Symptoms improved
11/19/37	9:30 a.m.	96	84	142.4	112.8	5.5	15.1	41.2	8.44	11.6		Appears normal

* Three cubic centimeters per kgm. cortical extract injected intravenously, and 3 cc. per kgm. administered daily in divided doses until recovery was complete.

† The M.L.D. for adrenalectomized dogs is somewhat less than 0.4 mgm./kgm. and approximately 0.73 mgm./kgm. for animals with intact adrenals.

TABLE 2—*Concluded*

DATE	TIME	BLOOD PRESSURE	PULSE PER MINUTE	SERUM SODIUM	SERUM CHLORIDE	SERUM POTASSIUM	HEMOGLOBIN	RED CELL VOLUME	R.B.C.	WEIGHT	DOSAGE PER KGM. WGT.	REMARKS
						No. 6 ♂						
	hours	*mm. Hg*		*m. eq. per l.*	*m. eq. per l.*	*m. eq. per l.*	*gm. per cent*	*per cent*	*millions*	*kgm.*	*mgm.*	
3/ 9/38	10:15 a.m.	104	80	140.4	111.8	5.4	10.7	31.5	4.68	12.9		Normal
3/ 9/38	10:55 a.m.	146	72								0.75	Adr. inj. 10:15–11:15
3/ 9/38	3:30 p.m.	52	180	145.5	114.0	5.2	11.1	36.6	4.76			Shock, extract*
3/10/38	10:15 a.m.	86	128									Much improved
3/12/38	9:30 a.m.	106	72	143.6	108.8	4.1	10.8	30.6	4.72	12.2		Normal
						No. 4 ♂						
11/23/37	9:40 a.m.	104	72	145.8	112.8	5.4	14.0	38.0	6.10	13.5		Normal
11/23/37	10:06 a.m.	144	88								0.75	Adr. inj. 9:40–10:45
11/23/37	5:40 p.m.	56	160	158.3	132.8	5.3	15.1	38.5	6.78			Shock, extract*
11/27/37	9:30 a.m.	100	96	140.6	110.8	4.3	14.6	39.3	6.08	12.8		Appears normal

blood concentration it is also evident that this type of shock is not primarily dependent upon decreased volume of fluid in circulation since the changes observed were not marked in any case. In general there was a tendency to concentrate the blood during shock but the degree of such concentration does not appear to be sufficient to induce symptoms.

In one case (dog 5, table 2) the blood was more concentrated after complete recovery from shock, on extract treatment, than it was when the animal was near prostration with the blood pressure at 56 mm. Hg.

In none of the experiments on either intact or adrenalectomized dogs were we able to correlate the shock symptoms and circulatory collapse with either hemoconcentration or serum electrolyte changes.

The data in table 2 show that cortical hormone when given in large doses intravenously, is as effective in reviving these animals from shock and restoring them to normal health and vigor, as it is in the shock and prostration of typical adrenal insufficiency. The condition of these dogs at the time of hormone injection precludes the possibility of their recovery from shock spontaneously. The experiments indicate that the restorative action of the hormone upon the circulation is separable and distinct from serum electrolyte changes and disturbances in the internal distribution of body water.

The effect of hypertonic saline was tested on two animals in adrenalin shock. A total of 40 cc. of 20 per cent NaCl solution was injected very slowly. The first 20 cc. were administered when the dog was in shock with the blood pressure at 46 to 48 mm. Hg. The pressure rose about 10 mm. Hg. Approximately 1 hour later a second injection of 20 cc. was given. At the end of 1 hour the arterial pressure was 62 mm. Hg but the symptoms did not appear to be much improved. Two hours from the time of the second injection the blood pressure began to decline although the hemoconcentration was reduced to near normal. Cortical extract was administered and the animal slowly returned to normal activity and vigor during the ensuing 36 hours. The arterial pressure, however, did not attain the normal level until the 4th day after injection of extract.

The time required to restore the collapsed circulation to normal by means of cortical hormone, varies surprisingly little for the various types of shock we have studied (muscle trauma, testis trauma, intestinal manipulation, adrenalin shock), where the active, vigorous, adrenalectomized dog was employed as the test animal. Three to four days is the average interval elapsing between shock and collapse, and complete recovery of the circulatory system. This statement also applies to recovery from uncomplicated adrenal insufficiency when the arterial pressure is permitted to decline to comparable levels. Occasionally the blood pressure is restored to normal within 30 hours after hormone administration, but this is unusual. Disappearance of symptoms and return of activity, vigor and appetite may occur within 18 to 24 hours but study of the blood pressure shows that it remains below normal (75–80 mm. Hg) despite the apparent recovery of the animal in other respects. In all of our experiments on shock, comparable dosage of hormone and method of administration have been used (Swingle and Parkins, 1935).

Elsewhere (Swingle, Parkins, Taylor and Hays—in press) evidence is presented strongly indicative of capillary atony, dilatation and stasis in adrenalectomized dogs following withdrawal of cortical hormone. The blood apparently accumulates in this greatly expanded peripheral reservoir and stagnates, so that free flow at the periphery becomes impossible and death from circulatory collapse occurs. Following hormone administration the capillaries regain tone, stasis slowly disappears and free blood flow throughout the periphery is reëstablished. The arterial pressure rises with the regain of peripheral tone and symptoms disappear.

Injecting massive doses of adrenalin into the healthy, vigorous adrenalectomized dog lacking hormone reserves, is merely another method of throwing a strain upon the circulation and thereby inducing shock. According to our interpretation, when any stress is thrown upon the circulation of this type of animal, the capillaries lose tone, dilate and circulatory failure results since insufficient cortical hormone is present to maintain tone of the capillaries or to regain tone once it is lost.

The intense visceral congestion and widespread hemorrhages invariably present in adrenalin shock, point to the vascular periphery and especially the capillaries, as the site of violent disturbance. Whatever the cause of death from adrenalin shock may be, we believe that the restorative action of cortical hormone upon the circulatory collapse is primarily through its effect upon the tonus of capillaries.

CONCLUSIONS

1. Massive doses of adrenalin injected intravenously at a constant rate produce fatal shock in the intact, unanesthetized dog. The M.L.D. is approximately 0.73 mgm./kgm.

2. Recovery from sublethal doses of adrenalin leads to the development of tolerance. The animals become highly resistant to subsequent doses, which are invariably fatal to dogs which have not been previously injected.

3. Changes in serum electrolytes, hemoconcentration or disturbances of the water balance of the body, are quite inadequate to account for the circulatory failure in this type of shock.

4. The evidence indicates that shock-inducing doses of adrenalin, among other things, cause violent disturbances at the vascular periphery and especially the capillaries. These appear to lose tone, and to dilate, thereby inducing general peripheral stasis and eventual circulatory collapse. We attribute the marked restorative action of cortical hormone upon the circulatory collapse in adrenalin shock to its effect upon the tonus of capillaries.

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EFFECTS OF CHEMICAL STIMULATION OF THE CAROTID BODY UPON THE REFLEX CONTRACTION OF THE TIBIALIS ANTICUS MUSCLE[1, 2, 3]

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Received for publication May 26, 1938

Within recent years much emphasis has been placed on the effects of presso- and chemo-receptor reflexes initiated at the carotid sinus region on diverse types of physiological activity (Heymans et al., 1933). For the presso-receptors it has been found that endo-sinusoidal hypertension inhibits reflex activity of striated muscle and decreases muscle tonus; whereas, endo-sinusoidal hypo-tension has the reverse effects (Tournade and Malmejac, 1929; Koch, 1932; Spychala, 1932; Mies, 1933; Heymans and Bouckaert, 1933; Tournade and Rocchisani, 1934). However, no analysis of the changes in reflex activity in response to chemo-receptor stimulation has come to our attention.

EXPERIMENTAL PROCEDURE. Dogs anesthetized with morphine (2 mgm./kgm.) and urethane (1 gm./kgm.) at a depth just sufficient to abolish the corneal reflex were used. The vago-sympathetic trunks were isolated for cold block. The trachea was connected directly to a system of rebreathing tanks designed to absorb carbon dioxide. Respiration was recorded by a spirometer directly connected with the rebreathing tanks; in addition, costal and abdominal respiratory movements were separately registered with the band pneumograph of Moyer and Gesell (1935). The latter procedure permits the registration of respiratory movements during pneumothorax. Down-stroke signifies inspiration; up-stroke, expiration.

With the exception of the internal carotid and the occipital arteries, the efferent vessels of the bifurcation of the common carotid artery were tied and cut between double ligatures, care being taken that Hering's nerve and the circulation to the carotid body were not injured. The lingual artery was cannulated so that backward intra-arterial injections could be made into the left innervated carotid body region. The cannula was

[1] A preliminary report of this paper appeared in the Proceedings of the American Physiological Society (1936), page 89.

[2] These experiments were supported in part by a grant from the Rockefeller Foundation to Robert Gesell for studies on respiration.

[3] A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the University of Michigan.

stitched to the adjoining muscular tissue to minimize mechanical stimulation of the carotid sinus region during backward intra-arterial injections. The right carotid body region was similarly dissected, but only after Hering's nerve had been identified and severed, and the internal carotids, occipital arteries, bifurcation of the common carotid artery, and common carotid artery (for a distance of 3 cm. below the bifurcation) stripped of their fascial investment. With this arrangement it could be determined whether or not changes in reflex contractions were of central or reflex origin.

The left sciatic nerve was isolated in the thigh; the branches to the hamstrings cut; the posterior tibial nerve identified, severed, and the central end placed on shielded electrodes of special design. Skin flaps were closed around the outer jacket of the shielding. The electrodes were connected to the secondary coil of an inductorium whose primary received the discharge from a thyratron stimulator. After immobilization of the left leg, the tendon of the tibialis anticus muscle was freed for a distance of about 6 cm. above its insertion, and connected by means of ligature to an isotonic spring-tension myograph. A slight constant tension was exerted on the muscle throughout the experiment. A fall of the base line of the reflex contractions indicated a decrease in the muscle tone; a rise, the converse. Freshly prepared solutions of M/20 sodium sulfide and M/100 sodium cyanide in 0.9 per cent saline diluted as necessary in normal saline were injected.

RESULTS. Backward intra-arterial injection (fig. 1, *F* and *C*) of 1 cc. of M/500 sodium cyanide or M/40 sodium sulfide (average injection time, at uniform rate, one second) into the innervated carotid artery produced noticeable changes in respiration, blood pressure, heart rate, and reflex contraction of the tibialis anticus muscle. Injections of 1 cc. of M/100 sodium cyanide or even of 1 cc. of M/20 sodium sulfide into the denervated sinus produced no changes. Control injections of 1 cc. of physiological saline into the innervated and denervated carotid sinus regions caused no perceptible effects. Since sodium cyanide and sulfide seemed to cause identical modifications in blood pressure, heart rate, etc. (fig. 1, *F* and *C*), sodium cyanide was used exclusively as the stimulating agent.

For economy of space, the phrase "stimulation of the chemically sensitive endings of the carotid body" has been shortened to "chemical stimulation"; "submaximal reflex contraction amplitude of the tibialis anticus muscle" has been shortened to "amplitude" or "reflex amplitude."

Modifications in the reflex amplitude. By varying the frequency of reflex stimulation through a range increasing progressively from 36 stimuli per minute to a value high enough to produce reflex tetanus, it was possible in most animals to obtain, as a result of chemical stimulation, a gamut of changes in the reflex contraction of the tibialis anticus muscle. For exam-

ple, at the rate of 36 reflex contractions per minute, in a naturally ventilated animal with intact vagi, chemical stimulation gave a diminution or no detectable decrease in the amplitude or tone. At the rate of 83 per minute (fig. 1, *B*), chemical stimulation caused a diminution in muscle tone together with a slight increase in the reflex amplitude. At 205 stimuli per minute (fig. 1, *G*), where a partial reflex tetanus had been established, chemical stimulation caused a greater reduction in the reflex tone with an associated increase in the amplitude of the discrete reflex contractions. Simultaneously undulatory changes in both tone and amplitude having a definite respiratory rhythm also appeared. With rates high enough to give reflex submaximal tetanus (fig. 1, *H*), chemical stimulation caused an abrupt, somewhat notched fall in the plateau. That this fall was not due to fatigue is shown by its absence in the plateau of reflex tetanus maintained for longer period without chemical stimulation. The latent period of the onset of alterations in tonus and reflex amplitude are similar to those of the corresponding hyperpnea and the duration of these changes (in all but the reflex submaximal tetanus) and the duration of the hyperpnea following chemical stimulation are of the same order.

Comparable results were obtained in vagotomized animals subjected to constant pulmonary ventilation during bilateral pneumothorax (fig. 1, *I*, *J*, *K*, *L*, *M*). At 180 twitches per minute, chemical stimulation produced two types of pronounced undulatory groupings in the reflex contractions which were independent of the rhythm of the artificial ventilation pump and related closely to the phases of respiratory movements. When at this rate there was no evidence that partial reflex tetanus existed, rhythmic changes occurred only in the reflex amplitude with the points of maximal amplitude at the beginnings of inspiration (fig. 1, *L*), the lowermost points at the beginning of expiration. However, when partial tetanus existed at this rate (fig. 1, *K*), fairly constant increased amplitudes accompanied wave-like tonic changes in which the crest occurred during inspiration and the trough corresponded to the beginning of expiration. At 390 reflex twitches per minute (fig. 1, *L*), the respiratory rhythm following chemical stimulation was less precisely defined than that for 180 stimuli per minute. In the pre- and post-stimulation portions of the records of reflex contraction at rates of 180 and 390 per minute, there is no hint of a respiratory rhythm in the configuration of the reflex twitches. These results before and after vagotomy classified in group one are by far the most representative.[4]

A sub-group of two animals merits special attention. Here, chemical

[4] In this particular animal with reflex submaximal tetanus (fig. 1, *M*), chemical stimulation produced a sharp stepwise rise (usual response, fall) in the plateau of reflex submaximal tetanus which is tentatively considered as "rebound" from an inhibitory volley.

RIS
LIS
A
1CC M/300 NaCN
1CC M/300 NaCN
10-24-35
B
IS VB
1CC M/500 NaCN
11-19-35
IS
B
1CC M/300 NaCN
IS VB
C
1CC M/40 NaS
DS
IS
1CC M/100 NaCN
D
1CC M/300 NaCN
IS VB
E
1CC M/40 NaS
IS VB
1CC M/500 NaCN
F
11-19-35
DS
IS
1CC M/100
G
1CC M/300 NaCN
NaCN
11-19-35
IS
NO PROCEDURE
H
1CC M/300 NaCN
10-31-35
VB IS
I
1CC M/300 NaCN
AB
CH
10-31-35
IS VB
J
DS VB
1CC M/300 NaCN
1CC M/100 NaCN
AB
CH
10-31-35
K
VB
1CC M/300 NaCN IS
10-31-35
390/MIN
L
VB IS
VB IS
1CC M/300 NaCN
1CC M/300 NaCN
AB
CH
10-31-35
M
VB IS
VB IS
1CC M/300 NaCN
1CC M/300 NaCN
AB
CH

Fig. 1

stimulation caused either a complete suppression of reflex contractions or else a marked decrease in reflex amplitude with little or no diminution in tonus with stimulation at frequencies of 36, 72, 90, and 132 per minute

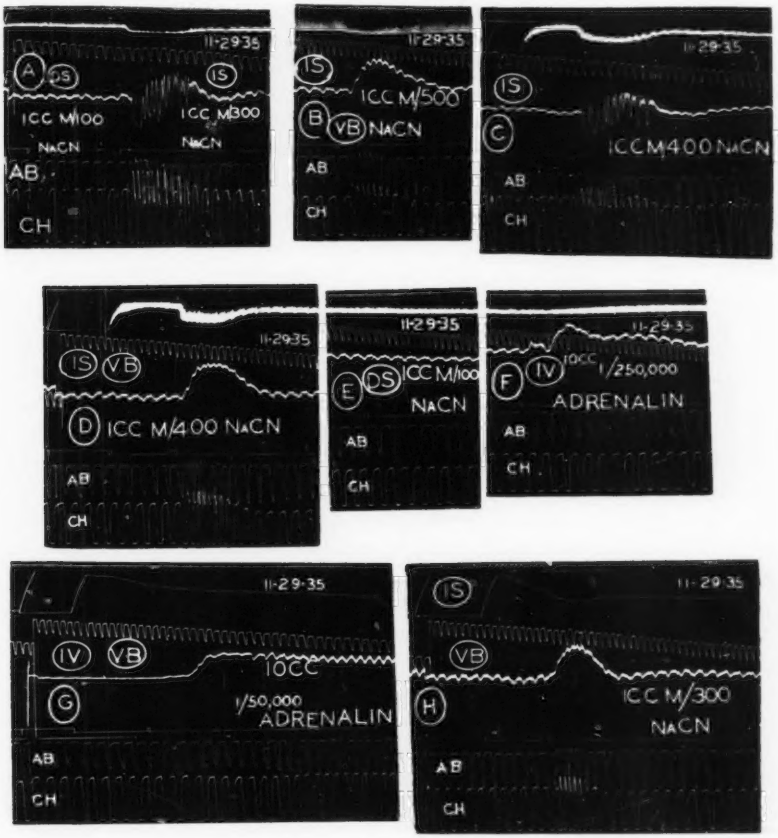

Fig. 2

Figs. 1 and 2. *IS* = injection into intact carotid sinus; *DS* = injection into denervated carotid sinus; *RIS* = injection into right intact carotid sinus; *LIS* = injection into left intact carotid sinus; *VB* = vagi blocked; *IV* = intravenous injection; *AB* = abdominal respiratory movements; *CH* = thoracic respiratory movements.

(fig. 2, *A*, *B*; fig. 1, *B″*). But when the rates increased beyond these values (fig. 2, *C*, *D*), the previously described diminution in muscle tonus occurred with either a diminution, no change, or actual augmentation of the reflex

amplitude, provided that the highest frequency producing discrete reflex muscle twitches was not exceeded. These results were obtained with the vagi intact or cold-blocked, with the animal normally or artificially ventilated. Carotid body stimulation produced in one of these animals notched depressions in the plateau of submaximal reflex tetanus (fig. 2, *H*). The notches occurred just prior to and practically synchronous with the beginning of each expiration.

Some animals, in the absence of experimental procedures, exhibited a well or poorly defined spontaneous respiratory rhythm of reflex amplitudes and of muscle tone. However, the changes in muscle tone were more regular than those of amplitude (fig. 1, *A*). The lowermost portion of the trough of the tonus wave coincided with the beginning of expiration; the beginning of inspiration fell on the descending portion of the tonus wave in advance of the point synchronous with expiration. The reflex amplitudes were lowest at the latter part of inspiration. In these animals, chemical stimulation intensified the first tonus wave by an exaggeration of all phases previously described (even to suppression of individual reflex contractions during inspiration) then reduced the tone, completely obliterating the undulatory base-line so prominent during the pre-stimulation period and simultaneously augmented the reflex amplitude.

In ten animals we were unable to show effects of chemical stimulation on reflex contraction. In three animals the tibialis anticus reflex could be elicited only with intense stimuli and following chemical stimulation no changes in reflex amplitude or muscle tone could be found. In seven dogs, the functional state of the vagi appeared to influence the changes resulting from chemical stimulation (King, Garrey and Bryan, 1932). In four of these animals changes in the reflex following chemical stimulation obtained only after the vagi were cold-blocked (fig. 1, *E*), the most typical findings being a poorly sustained, often abrupt, increase in the amplitude unaccompanied by tonic changes which occurred 10 to 30 seconds after the injection of sodium cyanide. In one animal subsequent to chemical stimulation there was a slight diminution both of the reflex amplitude and muscle tone (fig. 1, *C*, *F*) which was followed by a return of the tone to the pre-stimulation level and a long sustained period of increased reflex amplitude; further chemical stimulation reproduced these changes upon a background of higher reflex amplitudes. In two dogs subsequent to chemical stimulation, changes in the reflex amplitude occurred only when the vagi were functionally intact (fig. 1, *D*). Here the same latent period was occasionally observed both for the hyperpnea and the increased reflex amplitude (unaccompanied by tonic changes) following chemical stimulation; however, more often the latent period was one and a half to two times that of the accompanying hypernea. In these ten animals only the effects of chemical stimulation on the slow rates of reflex contraction were investigated be-

cause at the time these animals were studied the importance of frequency of the elicitation of the reflex twitch in relation to the alteration brought about by chemical stimulation was not realized. Furthermore, without exception, constant pulmonary ventilation was not employed in the study of these animals. In subsequent studies throughout which higher rates of reflex elicitation were employed, only two animals were encountered in which the effects of chemical stimulation on reflex contraction depended on the functional state of the vagi. These animals were very unstable and died soon after the experiment had begun.

Crossed nervous pathways. Chemical stimulation of the homo- or heterolateral carotid glands was equally effective in producing changes in the reflex contraction, indicating that the final central pathways mediating the changes in reflex contractions are composed of approximately equal numbers of crossed and uncrossed fibers (fig. 1, *A*).

Effects of changes in the systemic blood pressure. Since chemical stimulation produces variations in mean systemic blood pressure, capable of causing alterations in reflex contractions and tonus of striated musculature (Koch, 1932; Spychala, 1932; Mies, 1933), and since urethane anesthesia or any deep anesthesia abolishes tonus changes through presso-receptor reflexes our findings are related to the chemoreceptor stimulation only. This is supported by direct observation (see fig. 2, *B, D;* and fig. 2, *A, C*). In the first there is a rise and in the second a marked fall in pressure with no change in the reflex contraction in either. The method of Moissejeff (1927) in which the existing endo-sinusoidal pressures could be varied mechanically was also tried with the same result. Even doubling endo-sinusoidal pressure had no recognizable effect on reflex contraction despite the large fall in the systemic blood pressure. Elevation of the systemic blood pressure by intravenous injections of adrenalin (fig. 2, *F, G*) and occlusion and deocclusion of the common carotid arteries were without effect. Furthermore, vagal stimulation adequate to bring about cessation of the heart beat with a consequent fall of mean blood pressure to zero, produced no changes in reflex contractions.

DISCUSSION AND CONCLUSIONS. The changes in amplitude and tone of the tibialis anticus muscle can be explained upon a uniform basis, if one accepts that such stimulation causes showers of inhibitory nerve impulses to converge by crossed and uncrossed fiber tracts upon the anterior horn cells of the spinal cord, and that the inhibitory streams thus produced manifest themselves against a suitable background of excitation (Sherrington, 1906; Creed et al., 1932). To test the validity of this hypothesis let us first consider the tracing in which inhibition was pitted against reflex twitches of low frequencies (fig. 2, *A, B, C, D, H;* fig. 1, *B″*). It is assumed that immediately preceding chemical stimulation, the c.e.s. which causes contraction is built up in response to each electrical stimulus. Superposed

on these contractions, chemical stimulation caused an inhibitory barrage of nerve impulses to descend the spinal cord sufficient to inactivate part or all of the c.e.s. When no further inhibitory impulses descended in inactivating amounts, the c.e.s. within the motoneurones of the tibialis anticus muscle could build up once more to the nervous discharge level with the concomitant reappearance of reflex twitches. Diminution in the amplitude signifies that c.i.s. was produced in amounts great enough to prevent c.e.s. from reaching a threshold value in only a portion of the motoneurone field which responds to each shock of the stimulator.

When the rate of submaximal reflex contractions is increased, not only is the c.e.s. within the motoneurones built up at a faster rate, but also repetitive firing becomes a prominent feature. Although each stimulus to the afferent nerve still gives discrete reflex twitches, the degree of summation of the after-discharge often causes a considerable rise in the base line of the isotonic reflex contractions. At this stage the inhibition resulting from the chemical stimulation is most effective principally in the prevention of after-discharge. If the c.i.s. is strong enough, there is produced in addition to cessation of repetitive firing a decrease in the reflex amplitude due to inhibition of a portion of the previously active motoneurones. When this happens there is a diminution in amplitude upon a lowered tonic base line (fig. 2, *A*, *B*). With higher rates of stimulation (fig. 2, *C*, *D*; fig. 1, *G*), only the after-discharge is successfully inhibited because the waxing c.e.s. produced by the afferent excitatory volley is powerful enough to overcome the prevailing c.i.s. produced by chemical stimulation so that in response to this chemical stimulation there is either no change or more often an increase in the reflex amplitude upon a lowered tonic level.[5]

As far as we have been able to determine with control procedure, the effects of carotid body stimulation are in no way related to fatigue or

[5] The usual signs of inhibition following chemical stimulation were absent in more than 10 per cent of the animals in which under natural ventilation the effects of this stimulation were investigated at low rates of reflex contraction. This is not surprising since the effects of chemical stimulation cannot be demonstrated in most animals at these low rates of stimulation. However, in a portion of these animals increased reflex amplitudes were obtained in response to chemical stimulation after the vagi were blocked. This increased amplitude might have been caused by: 1, the blowing off of carbon dioxide; 2, increased sensitivity of the anterior horn cells in the vagotomized animal to excitatory stimuli; 3, the co-existence of visceral nervous factors such as those caused by a bladder distended with urine which makes its presence felt only after vagal block. Two naturally ventilated animals responded to chemical stimulation at low rates of reflex elicitation with increased amplitudes only when the vagi were intact; here it was felt that the blowing off of carbon dioxide was an important factor. No further statement concerning these animals is made. It is felt that the validity of the conclusions previously drawn on the basis of the behavior of the majority of animals is in no wise affected by the response of this smaller group to chemical stimulation.

volume flow of blood through cord or muscle. Complete inhibition of the heart for twenty seconds, or occlusion of the abdominal aorta as long as ten minutes caused no changes in reflex amplitude or tonus resembling those of chemical stimulation. Changes in tibialis anticus contraction must then be due to a reflex modification of the reflex process.

Irradiation from the respiratory center is a well recognized phenomenon as witnessed in the respiratory fluctuation of heart rate and blood pressure and of the knee jerk. Evidence points that this irradiation increases with the intensity of the respiratory act; for example, carotid body stimulation frequently gives rise to respiratory grouping of reflex contractions otherwise absent (fig. 1, *K*, *L*). These groupings are unrelated to the excursions of the lungs for in artificial respiration during pneumothorax they are in phase with the natural respiratory act. The groupings were more clearly defined during the deep breathing of vagal block (fig. 1, *K*) which is in agreement with the findings of Adrian, Bronk and Philips (1932) and King, Blair and Garrey (1931) (fig. 1, *L*; fig. 2, *H*). At rates high enough to produce reflex submaximal tetanus, chemical stimulation in some of the animals gave rise to descending inhibitory streams strong enough to inhibit this tetanus.

We come now to the relation of our findings to respiration. The carotid body is admittedly an organ of respiratory control which drives the respiratory center when oxidations are impaired. It accomplishes this end by increasing the rate of build up of the c.e.s. of the respiratory neurones thus increasing both rate and depth of breathing (Gesell, Steffenson and Brookhart, 1937). Excitation of the carotid body with sodium cyanide, if properly timed, is capable of augmenting a normal inspiration already on its way. A slightly later injection will augment an expiratory act (Gesell and White, 1937). This shows its direct connection with the respiratory mechanism. We now find that it is connected with motor system as a whole by its inhibitory action on the non-respiratory muscles. This reciprocal action of the chemoceptor organs raises the interesting speculation that they may play a part in respiratory control, particularly under conditions of stress, by giving precedence to respiratory reflexes.

SUMMARY

Effects of local excitation of the carotid body with sodium cyanide sufficient to cause marked augmentation of breathing, were studied on the reflex contraction of the m. tibialis anticus of the dog.

Such excitation at times reduced the amplitude of individual reflex contractions elicited at a low frequency of stimulation.

This same inhibitory action was more uniformly demonstrated on reflex and tonic contraction provoked by a higher frequency of stimulation.

These effects were not due to carotid sinus reflexes or to associated

changes in volume flow of blood or to fatigue. Their onset was synchronous with associated changes in breathing. They are tentatively explained on the assumption that descending inhibition interacts with the c.e.s. built up in the motor neurones of the tibialis anticus as a result of reflex stimulation.

It is suggested that the reciprocal action of the chemoceptors in building up c.e.s. in the respiratory neurones and in neutralizing c.e.s. in the neurones of the non-respiratory muscles may play a part in the control of breathing by giving precedence to respiratory reflexes. This may be an important factor during respiratory distress.

The writer wishes to thank Dr. Robert Gesell for his valuable suggestions and for his help.

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CENTRAL EFFECTS OF SODIUM SULFIDE UPON REFLEX CONTRACTION OF THE TIBIALIS ANTICUS MUSCLE[1, 2]

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Received for publication May 26, 1938

Gay (1929, 1930) found that the chief effect of intravenous administrations of sodium sulfide on the submaximal reflex contraction of the tibialis anticus muscle was an augmentation of the amplitude. Because this augmentation might have been due to a combined effect at the carotid and aortic body chemoreceptors and direct action on the reflex arc, it was thought desirable to re-investigate this problem under conditions which excluded the possibility of chemoreceptor stimulation.

EXPERIMENTAL. The preparation was similar to that employed in the preceding paper save that in all cases both carotid bodies were denervated and made non-responsive to test injections of sulfide. In addition, a few spinal animals were used with a transection level corresponding to the junction of the lowermost ribs with the vertebral column. Submaximal reflex stimulation of the tibialis anticus muscle occurring 40 to 60 times a minute were used, the chosen rate being maintained throughout the experiment. The solutions of sulfide were prepared before each experiment by dissolving dry sodium sulfide in normal saline.

RESULTS. The effects on the reflex amplitude depended on the amount injected and might be divided conveniently into four stages which are most clearly shown in the diagram. Essentially the same results were obtained in vagotomized and non-vagotomized animals, in naturally ventilated and in animals subjected to constant artificial ventilation after pneumothorax had been established. In spinal animals all stages described for sulfide action in non-spinal animals were accentuated.

Stage I. There is a slight but definite (rarely a large) augmentation of reflex amplitude with a subsequent return of the reflex to the pre-injection level. In normally ventilated animals the maximal swell bears no discernible relationship to events taking place in respiration as a result of

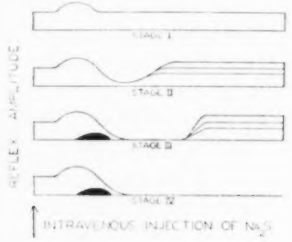
[1] A preliminary report of this paper appeared in the Proceedings of the American Physiological Society (1936), page 89.

[2] These experiments were supported in part by a grant from the Rockefeller Foundation to Robert Gesell for studies on respiration.

the sulfide injection; e.g., the period of maximum augmentation may come during the hyperpnea, after the hyperpnea, during the apnea, or after the apnea. The duration of the swell is usually short (10 to 20 sec.), but may be long (60 to 120 sec.). In either instance there is a return to the pre-injection reflex amplitude. Thus far, the findings are essentially those of Gay (1929, 1930).

Stage II. In this stage there may be a small or large initial increase of reflex amplitude followed by a definite decrease in the amplitude below the normal; which is in turn succeeded by reflex contractions which are either less than, equal to, or greater than the pre-injection level of reflexes. The latter instance is interesting since it represents a definite tendency to overshoot the level of the pre-injection reflex amplitude. The changes described bear no definite relationship to respiratory changes produced by sulfide injections in the normally ventilated animals.

Stage III. The initial increase of reflex amplitude may differ considerably from time to time and from animal to animal. The duration



of this phase is usually short. This augmentation is followed in turn by a phase characterized by decreased reflex amplitudes which finally reach a point where the depression is so profound that reflex contraction is no longer elicited. This period of reflex depression is succeeded by one during which the reflex arc is once more functioning, as is evidenced by the reappearance of the contractions of the flexor muscle. However, the first contractions are small; there is a gradual increase in the reflex amplitude; and the final level of the amplitude may be smaller than, equal to, or greater than that of the pre-injection level. In addition to changes described above, there may or may not be found a change in muscle tone during the latter part of the initial increase in the reflex amplitude which is continued into the first portion of the depression. This tonic change shall be considered in greater detail later.

Stage IV. A slight increase of amplitude is rapidly followed by a period of depression throughout which no reflex can be elicited with the same type of constant intermittent stimulation which during the pre-injection

period was adequate to bring about submaximal reflex contraction. This period of complete suppression of reflex activity is long-lasting and unless the intensity or the rate of stimulation is increased no reflex contraction can be elicited. Sometimes the slightest increase in intensity or rate is sufficient to cause an immediate return of reflex amplitude often greater than that of the pre-injection period. Once the reflex contraction has been reëstablished, the muscle will respond reflexly in the pre-injection way to the original stimulation. A sufficiently large dose of sulfide on the other hand causes a suppression of reflex contraction from which there is no recovery. After this stage is attained the animal usually dies of respiratory failure within from ten to thirty minutes; here irreparable damage is done to the central nervous system. In *stage IV* in addition, tonic changes in the tibialis anticus muscle may appear which are of the same nature as those indicated for *stage III*.[3]

Tonic changes due to sulfide injections. In the control period the tibialis anticus muscle responds without change in tone to each stimulus spaced from one to one and a half seconds apart. However, following sulfide injections, slight or marked increases in muscle tone lasting up to twenty seconds can be seen, during which time the summation of after-discharge continues from one to one and a half seconds after the initial afferent volley reaches the motoneurones as demonstrated by successive increases in the tonic level following successive stimuli. Usually only a portion of the muscle's moto-units is affected by the after-discharge, as is shown by the super-imposed reflex contractions in the response to the next succeeding intermittent stimulus against a background of increased tonus. There may be a gradual subsidence or an abrupt decrease of tonus to the pre-injection level. This tonic increase is almost always found only during the latter part of the first increase in reflex amplitude, especially in *stages III* and *IV*. At times this tonic change continues briefly into the succeeding period of diminished reflex contraction.

Discussion. A striking similarity exists between the response of the central respiratory mechanism (Winder and Winder, 1933) and the tibialis anticus reflex to sodium sulfide. They report a slight augmentation of respiration (minimal effects) which is analogous to *stage I* for the reflex contractions. They describe a depression preceded by a slight stimulation of respiration and followed by a recovery which might or might not be over-shooting (moderate effects) which closely resembles *stage II* for the reflex. *Stages III* and *IV* find their counterpart in what Winder and

[3] Occasionally, single sulfide injections cause a super-position of *stage IV* on the recovery period of *stage III*. In such instances either there is slow hydrolysis of the sodium sulfide or slow destruction of the hydrogen sulfide, or both, thus making it possible for the circulating blood to reach the reflex arc at two times, with enough sulfide dissolved to cause the double action.

Winder consider as the severe effects of central sulfide injections on respiration; i.e., *a*, apnea preceded by a stimulation of respiration and followed by recovery, and *b*, respiratory failure which might be preceded by stimulation of respiration. They also reported changes in the tonic level of the respiratory muscles which were paralleled by increases of the tibialis anticus muscle tone following intravenous sulfide injections.

The initial increased reflex amplitude subsequent to sulfide injections might be due to *a*, the engagement of more units for a given stimulus to the afferent nerve, or *b*, increased repetitive firing of the units already in action resulting from increased summation of subliminal after-discharge or *c*, a combination of these two effects. Considering the decreasing reflex amplitudes found in *stages II* and *III*, it can be seen that further sulfide action causes a gradual inability of more and more neurones to respond to afferent stimulation with a possible final result that all the motoneurones previously active become incapable of responding. Following this phase, there is gradual recovery which is the converse of the process of depression. However, if subsequent to sulfide injection there is complete suppression of the reflex amplitude for a constant stimulus without any indication of recovery, *stage IV*, then, an increase in the strength or rate of afferent stimulation (often slight) is adequate to cause the reappearance of reflex contractions at a level less than, equal to, or greater than that existing at the pre-injection level; this shows that increased rate of c.e.s. formation is effective in overcoming the suppression of reflexes caused by sulfide provided that there has been no permanent neuronal damage by sulfide. Therefore, the depression caused by sulfide might be due to an increased threshold for nervous discharge; prevention of c.e.s. formation in the motoneurones at a level permitting nervous discharge for normal thresholds; or the rapid dissolution and disappearance of c.e.s. so that the threshold level for discharge cannot be reached. Conceivably, all these factors can be operative simultaneously.

If neurones have the same power that blood has in sulfide detoxication, one might attribute the stimulating effect of sulfide (which is almost always the first recordable effect) to the entrance of unchanged hydrogen sulfide into the cell (Haggard, 1921; Haggard and Henderson, 1922). The depressing effect might be due either to the oxidation products of sulfides, or to the reduction products of intra-cellular substances formed by oxidation of sulfide. The duration and degree of depression would then be roughly proportional to the amount of sulfide oxidized. Spontaneous recovery from depression would indicate that the cell is capable of reëstablishing conditions as far as intra-cellular oxidation-reduction systems are concerned which approximate those existing at the pre-injection period. The tendency towards over-shooting the original pre-injection level would indicate that during recovery the intra-cellular

equilibrium is pushed far in the direction opposite that creating the depression. Paralysis of the reflex would indicate that if a great enough quantity of sulfide reaches the neurone, the establishment of the original conditions with autochthonous intra-cellular agencies is impossible; therefore, failure of neuronal function is permanent unless in some way a process such as increased c.e.s. can be inaugurated; or the intra-cellular products produced by sulfide action can diffuse into the blood stream; or substances tending to shift intra-cellular oxidation-reduction equilibria to the pre-injection level diffuse into the cell. However, overwhelming inundation of the nervous tissues by sulfide can cause irreversible neural damage.

The reflex arc of m. tibialis anticus is said to consist of a single afferent, efferent, and synaptic unit (Sherrington, 1906). The chief action of sulfide does not alter the contraction of directly stimulated muscle and only slightly increases the amplitude in muscle stimulated via its motor nerve. The tibialis anticus reflex is actuated by stimuli of constant strength coming at constant time intervals; the motor units of the reflex arc are therefore subjected to a periodic building up and discharge of c.e.s. which under control conditions manifests itself as fairly regular contractions. Similarly, in the master cells of the respiratory mechanism, there is a periodic waxing and waning of c.e.s. which under control conditions expresses itself as a fairly regular contraction with each respiration involving of necessity a phasic rise and fall in the c.e.s. of the anterior horn cells controlling the respiratory muscles (Adrian and Buytendijk, 1931; Gesell, et al., 1936).

The periodic waxing and waning of the c.e.s. of these cells can satisfactorily be accounted for by the electro-chemical membrane phenomena occurring at the boundaries of these master cells (Gesell, 1926). The building up of the c.e.s. within these neurones is conditioned by afferent streams of nerve impulses impinging, and the chemical state within the cells is conditioned by extra- and intra-cellular chemical factors. When the c.e.s. in these master cells reach the discharge level, they are capable of influencing the building up of c.e.s. in motoneurones innervating muscles of different segmental origin. The close parallelism between the changes in respiration due to central sulfide injections and the reaction of the simple reflex arc show a close similarity in the fundamental neural processes involved in both.

SUMMARY AND CONCLUSIONS

The m. tibialis anticus was activated reflexly by stimuli of constant strength uniformly spaced with respect to time. The reaction of the reflex arc to intravenous sodium sulfide injections was studied under conditions of natural and artificial ventilation in vagotomized and non-vagotomized animals, and in intact and spinal animals. The response to sulfide was similar in all the above preparations.

The description of sulfide action on the reflex arc has been divided into four stages. *Stage I:* slight increase in the reflex amplitude followed by the return to the pre-injection level. *Stage II:* depression of the reflex amplitude preceded by an initial increase in the reflex amplitude and followed by a period during which the reflex increases above the depression level to a value less than, equal to, or greater than the pre-injection level. *Stage III:* total suppression of reflexes as far as a stimulus of constant strength is concerned, preceded by an initial augmentation of the reflex amplitude; followed by a reappearance of the reflex contractions which are of gradually increasing amplitude and which may reach a value less than, equal to, or greater than that of the pre-injection level. *Stage IV:* here the initial increase of the reflex amplitude is followed by a permanent suppression of reflexes as far as the intermittent stimulus of constant strength is concerned. Changes in the tonus of the tibialis anticus muscle due to injection of sulfide have been pointed out.

A striking parallelism between the action of the reflex contraction of the tibialis anticus muscle to sulfide and the changes in respiration due to the central injections of sulfide reported by Winder and Winder is noted.

A possible mechanism for the action of sulfide on reflexes is pointed out.

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THE VITAMIN A RESERVE OF FUR-BEARING ANIMALS

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Received for publication May 28, 1938

The United States Department of Agriculture Bureau of Biological Survey (1937) reports that about 100,000 mink and 200,000 fox pelts are produced annually on ranches in the United States and that the valuation of fur produced on American-owned fur farms exceeds $9,000,000 annually. However, fur farming in this country is a new venture because this valuable industry has developed almost entirely since 1918. This phenomenal increase in fur farming has produced many problems concerning the nutritional requirements of fur bearing animals. Accordingly Federal Government research workers and commercial feed manufacturers are investigating various materials to develop more efficient rations, for the value of the fur is greatly influenced by the nutritive efficiency of the rations consumed by the fur bearing animals.

While few data are available concerning the vitamin A requirements of fur bearing animals, such data are extremely important in order to compound satisfactory rations. It has been assumed that vitamin A has the same importance in the growth, reproduction and well being of ranch raised, fur bearing animals as for other species of laboratory and domesticated animals. Early investigators such as Hopkins (1912), Osborne and Mendel (1913) and McCollum and Davis (1915) showed that vitamin A was essential for the growth of young animals and this conclusion has been repeatedly confirmed by many investigators. Also it was observed by Parkes and Drummond (1926), Evans (1928), Sure (1928), Hughes, Aubel and Lienhardt (1928), Hale (1935) and others that dietary deficiency of vitamin A causes a disturbance of the reproductive functions. On the basis of these data the rations of fur bearing animals must contain an adequate supply of vitamin A if satisfactory growth, reproduction and quality of fur are to be secured. In order to obtain information regarding the extent to which present rations fulfill these requirements this investigation was undertaken to determine the vitamin A reserve of several species of fur bearing animals.

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EXPERIMENTAL PROCEDURE. Livers for this study were obtained from New England fur farms when ranch raised, fur bearing animals were pelted. Whenever it was possible, data were assembled concerning the rations which had been consumed by the animals that produced the livers under consideration. In order to compare the vitamin A reserve of ranch raised animals with that of wild animals, livers from wild fur bearers were secured from various trappers upon their return from the trap line.

The livers were prepared for vitamin A assay by the method suggested by Torrance (1933) which consists of grinding the liver with an equal weight of anhydrous sodium sulphate. The amount of fat in the liver was determined by extracting a 20 gram portion of this mixture at 35°C. for eight hours with anhydrous ether. The remainder of the sodium sulphate-liver mixture was extracted for a short time with cold anhydrous ether. The fat obtained from the cold extraction was assayed for its vitamin A content by the antimony-trichloride colorimetric method of Carr and Price (1926) as modified by Lowen, Anderson and Harrison (1937). The antimony trichloride solution was prepared according to the method described in the British Pharmacopoeia (1932).

Mink livers. Twenty-seven mink livers were obtained from a Massachusetts fur farm when the animals were pelted in November. Practically all of the livers were taken from young animals that were born the previous spring. Livers from two wild mink are also included for comparison. The ration fed to the ranch raised mink consisted of about 80 per cent horse meat and fish scraps, and of 20 per cent commercial mink ration supplemented with fresh vegetables.

Data concerning the variations in body weight, liver weight, and fat content of the livers are reported in table 1.

The vitamin A content of the mink livers varied from 2.5 Lovibond Blue Units per gram of liver to 1,206.0 Lovibond Blue Units. The average vitamin A content of the livers from ranch raised mink was 62.0 Blue Units while that of the two wild mink was 975.0 Blue Units per gram of liver. The vitamin A stores of only two ranch raised mink approached the high vitamin A stores of the wild mink. In the absence of definite data it seems logical to explain the high vitamin A reserve of the wild mink to the fact that in the wild state the mink consumes the organs and viscera of fish and smaller animals which are a rich source of vitamin A. One mink, a $2\frac{1}{2}$ year old male, was judged to be sterile because of several unsuccessful matings during the previous spring. The vitamin A store of its liver was 11.8 Lovibond Blue Units per gram, while one other ranch raised mink which produced rough, poor quality fur, had a vitamin A store of 27.7 Lovibond Blue Units per gram of liver. Obviously the available data are too limited to permit satisfactorily comparing the vitamin A reserve of wild and domesticated mink, but they seem to indicate that the

TABLE 1

Vitamin A reserve of fur-bearing animals

ANIMAL		BODY WEIGHT			LIVER WEIGHT			LIVER WT. TO BODY WT.	LIVER FAT			L.B.U. PER LIVER			L.B.U. PER GRAM LIVER		
Kind	No.	Minimum*	Maximum*	Average*	Minimum	Maximum	Average		Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average
		grams	*grams*	*grams*	*grams*	*grams*	*grams*	*per cent*	*per cent*	*per cent*	*per cent*						
Mink (R)	27	375	800	499	22.5	58.6	34.7	6.95	2.00	7.56	4.27	61	36,000	2,515	2.5	614.4	62.0
Mink (W)	2	380	580	480	22.9	39.4	31.2	6.50	2.34	3.59	2.97	27,617	29,314	28,466	744.0	1206.0	975.0
Fox (R)	10	4500	6600	5200	134.4	236.0	183.8	3.53	1.75	3.47	2.25	202	906	457	1.5	4.3	2.5
Muskrat (W)	10	610	1023	844	16.1	50.8	30.5	3.61	2.48	8.62	4.51	1,385	7,525	4,589	39.8	281.4	165.9
Skunk (W)	10	966	4086†	1433	39.6	97.0	62.0	4.33	3.11	8.54	5.21	0	67,900	9,982	0.0	700.0	133.9
Opossum (W)	3	1589	1816	1740	77.0	135.5	99.1	5.70	4.45	6.15	5.15	10,469	22,290	17,529	123.6	257.5	181.9
Opossum (L)	2	1250	2500	1875	79.7	142.0	110.9	5.91	6.06	12.18	9.12	216,195	241,611	228,903	1522.5	3031.5	2277.0
Raccoon (R)	12	4994	9080	7001	74.1	160.0	110.1	1.57	3.57	7.86	5.08	0	0	0	0.0	0.0	0.0
Raccoon (W)	1						67.5				6.62			972			14.4
Rabbit (R)	5	1200	3178	2480	48.3	92.5	70.6	2.84	2.55	4.04	3.55	690	7,564	3,400	14.3	111.4	48.2
Rabbit (L)	5	720	4366	2601	28.2	148.0	82.8	3.18	3.04	4.29	3.91	649	10,778	4,751	21.6	126.8	52.9
Seal (W)	3								4.06	6.04	5.14				920.4	2228.7	1210.6
Bear (W)	1			128‡		*	2495.0	1.95			2.37			2,225,894			913.0
Weasel (W)	1			180			7.6	4.22			3.71			4,710			619.7
Squirrel (W)	3	384	550	469	9.7	11.1	10.5	2.24	4.06	5.26	4.80	132	1,558	1,011	11.9	160.0	99.7

* Body weights for mink, muskrats, raccoons, weasel and skunks represent the dead animal with pelt removed.

† Body weight of skunk with pelt.

‡ Kilograms.

R-Ranch.

W-Wild.

L-Laboratory.

vitamin A content of commercial mink rations is too low to permit domesticated mink to accumulate an optimum vitamin A reserve.

Fox livers. Ten fox livers were obtained in December from the same fur farm as the mink livers. Four of the animals were young foxes that were born the previous spring. Two of the foxes were old females. It is interesting to note in this connection that one of the old females showed the highest reserve, 4.3 Blue Units per gram of liver. The young foxes gave values ranging from 1.9 Blue Units to 3.8 Blue Units per gram of liver. The blue value for the ten foxes varied from 1.5 to 4.3 Lovibond Blue Units per gram of liver, the average being 2.5 Blue Units.

The foxes had been fed a ration which consisted of 25 per cent to 30 per cent of a commercially manufactured fox ration and the remaining 70 per cent to 75 per cent consisted of horse meat and fish scraps and occasionally some miscellaneous vegetables. The commercially manufactured fox ration was reported to contain dried liver, fish, beef, buttermilk, skimmilk, vegetables, cereals, wheat germ meal, fish oil, molasses, bone meal and minerals.

The data concerning the body weight, liver weight, and fat content of the livers are summarized in table 1.

It is interesting to note that the vitamin A content of fox livers, 2.5 Lovibond Blue Units per gram of liver, is very much less than 62.0 Lovibond Blue Units per gram of liver which was obtained for the mink livers. In this connection it may be noted that Smith (1935) reports that foxes do not require large amounts of vitamins A and D during the summer, fall and premating seasons. On the other hand he states that vitamins A and D are very important and should be fed during the gestation period.

Muskrat livers. Ten muskrat carcasses, with the pelts removed, were secured from fur trappers in the vicinity of Cape Cod, Massachusetts. The variations in the carcass weights, liver weights, and percentages of fat in the livers are recorded in table 1.

The vitamin A content of the muskrat livers varied from 39.8 to 281.4 Blue Units per gram of liver. The average value for the ten muskrats was 165.9 Blue Units per gram of liver. This value, 165.9 units, for animals which subsist largely on vegetable material, is very much higher than the vitamin A reserve reported above for foxes and mink that consumed generous amounts of animal material. It may be concluded from these data that the fox and mink rations were not good sources of vitamin A.

Skunk livers. The ten skunk livers were obtained from several trappers in the vicinity of Cape Cod, Massachusetts, during the months of January and February.

The average vitamin A content of the ten livers was 133.9 Lovibond Blue Units per gram of liver. A study of table 1 will reveal a wide variation in the vitamin A stores of the ten skunks under consideration. It

seems quite evident from this observation that the abundance and vitamin quality of the food of skunks in the wild state fluctuates over wide limits.

No vitamin A could be found in the livers of two male skunks, while a relatively high vitamin A reserve, 700 Blue Units per gram of liver, was found in the liver of another male animal.

Opossum livers. Five mature opossums, four males and one female, were obtained from hunters and trappers located in Virginia and North Carolina. All of the opossums had spent their entire lives at liberty under natural conditions. Three of the animals were killed on arrival at the laboratory. Two were kept for a period of six months and received a ration consisting of the organs of rats and chickens, beef, hog, calf and lamb livers, eggs, milk, carrots and other vegetables. The beef, hog, calf and lamb livers fed to the opossums were portions of the forty-six livers whose vitamin A content was reported by Holmes, Tripp and Satterfield (1936).

Data concerning the variations in body weight, liver weight and percentage of fat in the livers are reported in table 1.

The vitamin A stores of the three opossums, which were killed as soon as obtained from the wild state, varied from 123.6 Blue Units to 257.5 Blue Units per gram of liver or an average of 181.9 Blue Units which may be assumed to be representative of the vitamin A reserve of wild opossums. On the other hand the vitamin A stores of 1,522.5 and 3,031.5 Blue Units per gram of liver for the two opossums confined in the laboratory for a six months period represents the capacity of opossums for storing vitamin A when provided with a vitamin A rich ration.

Raccoon livers The raccoon livers were obtained from a Vermont fur farm when the animals were pelted in December but it should be noted that the animals had hibernated a short time before they were killed. The raccoons were raised on a diet which consisted of the commercially manufactured fox ration described above, supplemented with apples.

The raccoon livers were different from the livers obtained from the other fur bearing animals in that they were very hard, tough, rubber-like and difficult to grind. The fat obtained from these livers was also very different from the liver fat of the other species of fur bearing animals. The raccoon liver fat varied from a reddish brown to a black tar-like color whereas the liver fat from the other fur bearing animals was pale yellow to light brown in color. No vitamin A could be detected in the fat from any of the twelve raccoon livers. Subsequently the twelve fats were combined and saponified. The unsaponifiable portion was tested for its vitamin A potency but none could be found.

A liver from a wild raccoon was obtained from a New Hampshire trapper. The appearance of the liver and liver fat from this raccoon was similar to that of the twelve ranch raised raccoons. The liver was assayed

for its vitamin A potency but only a small amount, 14.4 Lovibond Blue Units per gram of liver, was found.

Table 1 summarizes the data obtained for the raccons concerning the body weight, liver weight and liver fat.

Rabbit livers. Ten rabbit livers were analyzed for their vitamin A content. Five of the livers were obtained from New Zealand White rabbits which were raised in the laboratory. The other five rabbits were procured from nearby farms in order to compare the vitamin A reserve of their livers with that of the laboratory animals. The ration of the laboratory raised rabbits consisted of commercially manufactured rabbit pellets supplemented with carrots and some green vegetables.

The vitamin A reserve of the ten rabbits varied from 14.3 Blue Units per gram of liver for a mature farm grown Flemish Giant to 126.8 Blue Units per gram of liver for an adult New Zealand maintained for one year under laboratory conditions. Two one month old rabbits had a vitamin A store of 23.0 and 22.3 Blue Units per gram of liver respectively.

The average vitamin A reserve for all the ten rabbits was 50.5 Blue Units per gram of liver. It is interesting to compare this value, 50.5 Blue Units per gram of liver, obtained for rabbits grown in captivity with 165.9 Blue Units obtained for wild muskrats which also subsist on vegetable material. In the absence of data to the contrary it appears that the rabbit rations did not contain a sufficient supply of vitamin A to enable the rabbits to accumulate optimum stores of vitamin A.

Seal livers. Three composite samples of frozen seal livers were obtained from Newfoundland. These samples represented bedlamer, female and male seals and all the livers were chocolate colored. The Newfoundland seals are not used as a source of fur, but instead their hides are used for the manufacture of leather. The bedlamer seals are young male seals weighing about 150 pounds and varying from one to three years in age. It was reported that the livers from the male and female seals were taken from animals which were more than three years of age. Unfortunately no data were supplied concerning the weights of the seals or of their livers.

The fat content of the livers was 4.06 per cent for the bedlamer seal livers, 5.32 per cent for the female seal livers and 6.04 per cent for the male seals.

The vitamin A content of the three types of composite livers was 482.8 Blue Units per gram of liver for the bedlamer seal livers, 920.4 Blue Units for the livers from the females and 2,228.7 Blue Units for the livers from the male seals.

The seal liver fats were also tested by the biological U. S. P. XI (1936) method. It was found that the fat from the bedlamer seal livers contained less than 25,000 Vitamin A units per gram of fat, while the liver fats from the male and female seals contained over 25,000 U. S. P. XI Vitamin A units per gram of liver fat.

The seal liver fats were also assayed by the Carr and Price (1926) antimony trichloride colorimetric method. The liver fat from the bedlamer seals contained 11,891 Blue Units, the liver fat from the female seals 17,301 Blue Units and the liver fat from the male seals contained 36,900 Blue Units per gram of liver fat. It is evident from these observations that the rations of the wild Newfoundland seals contain sufficient vitamin A to permit the seals to accumulate large reserves of vitamin A.

Bear liver. The liver was obtained from a 128 kilogram black bear killed in the White Mountain area of New Hampshire in September. No information was obtained concerning this animal's ration.

The vitamin A content of the liver was found to be 913.0 Blue Units per gram. The liver fat assayed by the Carr-Price method had a potency of 38,462 vitamin A units per gram and assayed by the U. S. P. biological method 75,000 vitamin A units per gram. These data supply quite definite evidence that the ration of the wild black bear contained generous amounts of vitamin A.

Weasel liver. The liver from a white weasel was procured from a Southeastern Massachusetts trapper. Since the weasel had grown under natural conditions its food undoubtedly included liberal amounts of animal material. The analysis of the liver showed it to have a vitamin A potency of 619.7 Blue Units per gram. This vitamin A store compares very favorably with that of wild mink, skunk and opossum, all of which have food habits similar to those of the weasel.

Squirrel livers. Three wild gray squirrels were obtained in Eastern Massachusetts. The livers showed wide variation in vitamin A potency. The minimum store of vitamin A was only 11.9 Lovibond Blue Units per gram of liver for one of the females. The second female had a store of 127.2 Blue Units and the male a store of 160.0 Blue Units per gram of liver. It is evident from these observations that squirrels have a capacity for storing considerable amounts of vitamin A when their natural food supply contains an adequate quantity of this vitamin.

SUMMARY

A study has been made of the vitamin A reserve of eleven species of fur bearing animals. The vitamin stores were judged by the results of the assay of livers by a modified Carr-Price colorimetric method.

The vitamin A potency of livers from animals raised in captivity was 62.0 Blue Units for mink, 2.5 units for foxes, 0.0 unit for raccoons, 48.2 and 52.9 Blue Units per gram of liver for rabbits. Compared with these low values the vitamin A potency of animals grown under natural conditions was 975.0 Blue Units for mink, 165.9 units for muskrat, 133.9 units for skunk, 181.9 units for opossum, 14.4 units for raccoon, 1,210.6 units for seal, 913.0 units for bear, 619.7 units for weasel and 99.7 Blue Units per gram of liver for squirrels. A comparison of the vitamin A

reserve of 181.9 Blue Units per gram of liver for wild opossums with 2,277.0 Blue Units per gram of liver for opossums fed a vitamin A rich ration under laboratory conditions for six months, indicates the possible capacity of fur bearing animals for storing vitamin A. A consideration of various comparisons of the vitamin A reserve of ranch raised and wild animals indicates that the rations of ranch raised animals did not contain sufficient vitamin A to enable the animals to accumulate optimum reserves of this vitamin.

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BLOOD-CHEMICAL AND OTHER CONDITIONS IN NORMAL AND ADRENALECTOMIZED SLOTHS[1]

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Received for publication June 1, 1938

Comparative mammalian studies have led through the years to a rich understanding of man morphologically; yet within only a few years past have similar studies been pursued toward a functional appreciation of life. It is readily admitted that from considerations of comparative physiology, however, are likely to come highly important fundamental results affecting many phases of human physiology. In quest of answers to the adrenal enigma we have already utilized a wide variety of species—rat, guinea pig, rabbit, cat, dog, squirrel, opossum, marmot, etc.—and recently we have included a number of primitive and primate mammalian forms. A very lowly type, the sloth, is singularly interesting because of its proximity in some important aspects to the reptilian type (Britton and Atkinson, 1938), while there are other evidences of its affinity to the primates (Wislocki, 1927; Heuser and Wislocki, 1935). In the past two years we have studied adrenal insufficiency and normal conditions in the common Central American sloths, the two-toed *Choloepus hoffmanni* and the three-toed *Bradypus griseus*. Although these two types are strikingly different in many anatomical and physiological respects, and in behavior the latter is much the simpler, their reactions to adrenal removal were throughout rather uniform.

Adult male and female sloths were used. Adrenal removal was carried out in a one-stage operation lasting 5 to 10 minutes, without sterile precautions. Blood and tissues were taken from both normal and operated animals after stunning, in the non-fasting state. The adrenalectomized sloths were utilized only after symptoms had become evident. The physiological investigations were carried out in Central America, and

[1] The physiological phases of these studies were carried out at the Barro Colorado Island Laboratory, C. Z., and the Gorgas Memorial Laboratory, Rep. Panama, under the terms of a John Simon Guggenheim Memorial Fellowship held by the senior author during 1937–1938. The thanks of the authors are gratefully extended to Mr. James Zetek and Dr. Herbert C. Clark of these laboratories, respectively, for their unfailing courtesies during the investigations.

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animals were kept in large open-air cages adjacent to the jungle, and given their natural diet of cecropia leaves and tropical fruits. Most of the chemical analyses were made at the University of Virginia according to the method described in the previous paper (Britton, Silvette and Kline, 1938).

After adrenal removal the behavior of sloths differed very little from normal for about 12 hours. Thereafter activity became progressively less than usual, and most animals were at the point of death within only 24 hours after the time of operation. This extremely short survival was in contrast to several days' survival after bilateral nephrectomy, and other observations (unpublished results) that the sloth is extremely hardy and holds tenaciously to life even after severe trauma.

It was usually very difficult to decide whether an animal had actually succumbed or not after adrenal removal. In some instances very slow flexion and extension movements of all four limbs, and dorsiflexion of the head, were observed towards the end of the period of insufficiency. These movements were rather suggestive of convulsive seizures—such as might be expected in an organism like the sloth. Paralysis of all limbs was observed terminally, and at death flexion of the claws disappeared and the sloth fell to the ground. Large quantities of food materials were always found in the stomach and intestinal tract at autopsy.

Analytical results are given in synopsized form in table 1. Values for normal serum sodium and chlorides and serum sugar and liver glycogen were lower than we have observed in other mammals. In the case of liver glycogen particularly the levels found in over 20 non-fasting sloths were only about 30 per cent of the mammalian normal. Serum potassium and urea readings were not significantly different from those found in other mammals.

After adrenal removal it will be observed that serum sodium and chloride levels were hardly affected, although a tendency toward reduction was usual. Serum potassium was definitely increased, and also serum urea. Serum sugar and liver glycogen values appeared to be most affected, there being reductions in both cases, and in both animal species, of approximately 50 per cent. It would seem likely that these levels would be incompatible with life in the sloth. Muscle and heart glycogen levels were also considerably reduced.

Some recent observations which we have made on sloths are possibly explanatory of the low levels of liver glycogen found in normal non-fasting animals. Thus, this lowly mammalian type possesses only about one-half the amount of skeletal muscle tissue found in higher mammals—about 25 per cent only of the body weight compared to about 45 per cent in animals such as cats, dogs, etc. Again, sloths show a relatively low normal body temperature—3° to 4° below that of the higher mammals. The extraordinarily small amount of muscular activity, and that of a very simple type, which is undertaken by the sloth in its usual daily round, is

also probably correlated with (low-grade?) carbohydrate metabolism in this organism. Also, sleeping or resting about 20 hours out of the day, the sloth performs perhaps no more than 10 per cent of the work of a higher mammal of the same size. The liver is moreover very small—about one-half the size of that found in other animals—while the stomach (with contents) is very large—about 25 per cent of the body weight. After several days' fasting the stomach of the sloth was found to be still approximately half-full. Probably a fairly continuous but small supply

TABLE 1

Carbohydrate, salt and other blood and tissue levels in normal adrenalectomized sloths

EXPERIMENTAL CONDITION		SURVIVAL	SERUM					GLYCOGEN			MUSCLE WATER
			Sodium	Potassium	Chlorides	Urea	Sugar	Liver	Muscle	Heart	
Two-toed sloths (*Choloepus hoffmanni*)											
		hours	*mE per l.*	*mE per l.*	*mE per l.*	*mgm. per cent*	*mgm. per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Normal (10 animals)	Minimum		122.1	5.82	92.8	64	59	0.23	0.22	0.29	73.1
	Maximum		141.8	10.3	103.8	166	137	0.72	0.72	0.71	75.6
	Average		131.0	7.73	99.7	106	88	0.50	0.42	0.48	74.7
Adrenalectomized (11 animals)	Minimum	12	111.9	9.02	88.5	88	48	0.17	0.16	0.15	73.9
	Maximum	38	147.4	12.3	103.0	161	70	0.39	0.60	0.81	79.4
	Average	24	125.4	10.7	95.9	134	54	0.25	0.31	0.38	75.9
Three-toed sloths (*Bradypus griseus*)											
Normal (13 animals)	Minimum		118.1	3.59	94.8	53	63	0.30	0.23	0.28	70.0
	Maximum		142.4	10.3	110.8	129	100	0.98	0.69	0.95	77.7
	Average		128.2	5.72	101.8	81	84	0.54	0.47	0.61	74.7
Adrenalectomized (14 animals)	Minimum	20	117.8	6.83	91.6	64	19	0.05	0.06	0.06	69.0
	Maximum	58	137.0	11.6	109.8	202	51	0.50	0.63	0.66	77.2
	Average	28	131.0	9.90	100.8	112	35	0.23	0.36	0.37	73.0

of glucose would therefore be available to the hepatic tissues for glycogen deposition.

The extreme functional limitations found in the sloth may thus be somewhat better understood from the observations above. That this animal is greatly dependent on its adrenal mechanism is also apparent from its very brief post-adrenalectomy survival period—the briefest which we have encountered in any animal used. There is possibly some correlation between the latter fact and the low normal glycogen reserves found in the sloth.

SUMMARY

Normal non-fasting two-toed and three-toed sloths (*Choloepus hoffmanni* and *Bradypus griseus*) show very much lower hepatic glycogen reserves than are found in higher mammalian types. Serum sodium and chloride and serum sugar are also somewhat lower.

Sloths possess only about one-half the amount of muscle (by percentage) found in higher forms, and are probably no more than one-tenth as active. Normal body temperature levels are 3° to 4° lower. The liver is moreover only about one-half the size of that found in other mammalian types.

After adrenal removal blood-chemical and tissue conditions in the sloth show changes which are generally in the same direction as those found in higher types. Serum glucose and liver glycogen are greatly reduced to levels which would appear to be incompatible with life. In correlation muscle and cardiac glycogen values in adrenal insufficiency were also markedly lowered.

Considering the extremely short survival of the adrenalectomized sloth—about 26 hours only on the average, which is much shorter than that found in any other animal to date—as well as its remarkable lethargy, which is more apparent after operation, the foregoing observations on carbohydrate reductions are more emphatically significant.

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ADRENAL INSUFFICIENCY IN AMERICAN MONKEYS[1]

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Received for publication June 1, 1938

While numerous experiments on adrenal function have been carried out on lower mammals and even on sub-mammalian forms, the primates have hardly been considered at all in this field of study. It would appear, however, that observations on adrenal insufficiency in monkeys might add more particularly to our knowledge of human adrenal physiology, and also shed fundamental light on the clinical condition of Addison's disease in man. Opportunity was recently afforded for studying, in their native habitat, three common species of American monkeys, viz., the capuchin or white-faced monkey, *Cebus capucinus imitator* Thomas; the night-monkey, *Aotus zonalis* Goldman; and the marmoset or squirrel-monkey, *Leontocebus geoffroyi* Pucheran. In these animals observations on adrenal insufficiency proved to be particularly interesting.

METHODS. The animals used were kept in large open-air cages, and frequently other monkeys from the adjacent jungle (Barro Colorado Island) came by and chattered with the experimental group. A complete diet of mixed tropical fruits, with occasional small amounts of meat relished by these monkeys, was given. Adult or near-adult males and females were used. The adrenals were easily accessible and were removed with very little trauma in one operation under ether, lasting only 5 to 10 minutes. No sterile precautions were used in most cases, since it was found that ordinary cleanliness alone gave good (non-infective) operative results in work in the tropics. Normal and operated animals were utilized in the non-fasting state.

Protein-free filtrates of heparinized plasma were prepared by adding 3 volumes of distilled water and 1 volume of 20 per cent trichloracetic acid to 1 volume of plasma. After standing and filtration the trichloracetic acid filtrates were stored in glass-stoppered Pyrex bottles. Sodium

[1] The physiological phases of these studies were carried out at the Barro Colorado Island Laboratory, C. Z., and the Gorgas Memorial Laboratory, Rep. Panama, under the terms of a John Simon Guggenheim Memorial Fellowship held by the senior author during 1937–1938. The thanks of the authors are gratefully extended to Mr. James Zetek and Dr. Herbert C. Clark of these laboratories, respectively, for their unfailing courtesies during the investigations.

[2] E. R. Squibb and Sons Fellow in Physiology.

(Butler and Tuthill, 1931) and potassium (Kramer and Gittelman, 1926) analyses were made on 5 cc. quantities of plasma filtrate evaporated to dryness with a drop of nitric acid and redissolved in 1 cc. of distilled water. Chloride and urea determinations were made directly on the trichloracetic acid filtrates, the former by the method of Van Slyke and Sendroy (1923) and the latter by the gasometric procedure of Van Slyke and Kugel (1933). Plasma glucose determinations were made by the method of Folin and Malmros (1929) on carefully neutralized aliquots of the protein-free filtrates.

Portions of muscle tissue weighing about 1 gram were placed in previously weighed small tubes containing 4 per cent formaldehyde. Later, the tubes were again weighed to give by difference the weight of fresh tissue, and the contents poured into aluminum weighing-pans of known weight and placed in an oven at 105° until constant weight was attained. The added formaldehyde solution evaporated off completely, as did the muscle water, and the true water content of the muscle was thus determined.

Tissue glycogen determinations were made by the Pflüger procedure modified for small amounts of tissue (Silvette and Britton, 1932). Control determinations showed that after solution of the tissue had taken place in the hot alkali, no glycogen loss occurred even though the alkaline glycogen mixture stood for several weeks.

Results. Exceedingly high liver glycogen levels, even for animals which were digesting food, were commonly found in the normal monkeys used. Several cases varied from 8 to almost 12 per cent (table 1). The averages were: capuchins 2.8, night-monkeys 6.6, and marmosets 2.4 per cent. In this connection it was found that at the time of sampling, the liver tissues were strikingly glutinous to touch. Plasma glucose and muscle and heart glycogen levels in normal monkeys did not differ greatly from those found in man and many other animals. Similarly, serum sodium and chloride and muscle water were comparable.

Within a short while after adrenal removal, food was taken readily and the usual playful and other responses appeared normal. After a few days or a week or so, insufficiency symptoms of lethargy and weakness began to appear, and continued to progress gradually for one or two days. Even up to the time and during the period of prostration, however, food—especially bananas—would often be taken. Convulsions invariably supervened and were of the strikingly typical hypoglycemic type—tonico-clonic spasms, clawing, turning, limb extension, dorsiflexion, etc. They frequently did not appear until an animal had been prostrated for several hours. At first they were observed about once an hour or so, and then gradually became more frequent until after about six hours they occurred once in 15 to 20 minutes. Convulsive cries and increased salivation were

TABLE 1

Carbohydrate, salt and other blood and tissue levels in normal and adrenalectomized new-world monkeys

ANIMAL NUMBER	SEX	EXPERIMENTAL CONDITION	SURVIVAL	SERUM				GLYCOGEN			MUSCLE WATER
				Sodium	Chlorides	Urea	Sugar	Liver	Muscle	Heart	
					White-faced monkey (*Cebus capucinus imitator* Thomas)						
			hours	*mE per l.*	*mE per l.*	*mgm. per cent*	*mgm. per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	F	Normal		140.4	110.4	88	114	1.08	0.51	0.56	73.2
2	F	Normal		143.8	114.6	65	89	1.81	0.72	0.60	75.2
3	F	Normal		150.1	117.2	52	133	1.46	0.61	0.48	72.9
4	F	Normal		139.1	115.0	54	69	0.57	0.69	0.60	74.9
5	F	Normal		136.5	110.2	139	111	9.55	0.42	0.36	76.5
6	F	Normal		142.4	122.8	126	109	1.52	0.78	0.47	74.1
7	F	Normal		150.8	112.8	142	117	1.32	0.75	0.34	74.9
11	M	Adrex†	187	124.2	98.6	132	21	0.13	0.51	0.22	76.1
12	M	Adrex	48	122.9	106.0	142	18	0.07	0.48	0.18	76.2
13	M	Adrex	126	125.5	104.6	103	32	0.10	0.41	0.20	76.2
14	M	Adrex	437	126.8	103.2	175	30	0.12	0.20	0.02	75.0
15	F	Adrex	25	130.7	110.6	156	33	0.21	0.56	0.14	76.0
					Night monkey (*Aotus zonalis* Goldman)						
1	F	Normal		122.2	99.6	118	144	1.41	0.61	0.16	73.6
2	F	Normal		137.2	106.6	130	114	2.75	0.42	0.26	74.6
3	F	Normal		139.1	112.4	127	100	8.75	0.69	0.25	74.9
4	F	Normal		147.2		88	122	3.34			75.4
5	M	Normal		144.3	113.8	87	111	11.8	1.27	0.32	74.4
6	F	Normal		146.3	117.2	84	125	11.6	1.27	0.36	75.0
11	M	Adrex	160	126.9		118	64	0.13	0.29	0.19	72.3
12	M	Adrex	194	133.9		208	46	0.09	0.35	0.27	74.2
13	F	Adrex	21	137.8	98.8	165	163*	0.22	0.42	0.30	73.5
14	M	Adrex	78	134.6		91	127*	0.18	0.32	0.08	68.8
					Marmoset (*Leontocebus geoffroyi* Pucheran)						
1	F	Normal		158.8	113.2	113	109	3.77	0.52	0.18	71.9
2	F	Normal		143.7		86	80	1.71	0.15	0.33	73.8
3	M	Normal		158.0	120.6	109	106	1.61	0.96	0.46	76.3
11	M	Adrex	63					0.21	0.25	0.09	75.5
12	M	Adrex	69	136.5		191	31	0.13	0.44	0.25	74.1
13	M	Adrex	27	150.2	112.4	179	29	0.19	0.31	0.24	74.8

* These high figures are probably explained by the very short survivals after operation.

† Adrenalectomized.

observed on several occasions. In the last stages of insufficiency the seizures often tended to become continuous, and sometimes ended in convulsions of an obviously different, asphyxial character. Many of the adrenalectomized animals were sampled shortly after convulsions had set in; in a few cases they were utilized at the point of death.

Extremely low serum sugar and liver glycogen values were found in monkeys suffering from adrenal insufficiency. Considering all series (see footnote exception, table 1), the average reduction of serum sugar from normal was over 70 per cent (9 cases), while liver glycogen was reduced over 90 per cent (12 cases). Muscle and heart glycogen values were also greatly reduced following adrenalectomy (see averages, fig. 1).

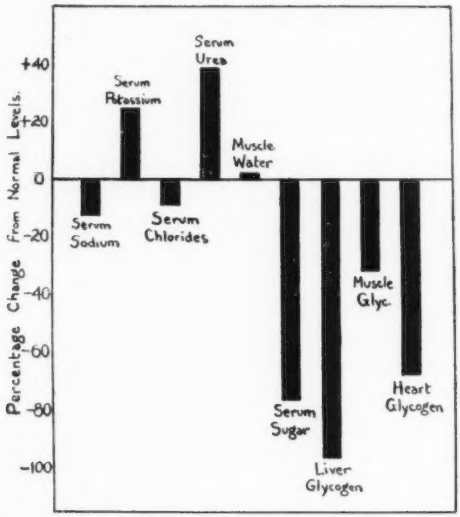

Fig. 1. Blood and tissue changes in the white-faced monkey after adrenalectomy

It is worthy of note, however, that food was often found in the stomach, and food residues almost invariably in the small intestines, in the adrenalectomized monkey at autopsy.

Decreases in serum sodium and chlorides, and increases in serum urea and potassium, were observed in adrenal insufficiency in monkeys, in keeping with data derived from other animal types. Also, muscle water usually tended to show an increase.

The survival periods after adrenalectomy were variable, and ranged from 1–18 days in capuchins (average 7), 1 to 8 days in night-monkeys (average 5), and 1 to 3 days in marmosets (average 2).

Discussion. The small amount of adrenal work hitherto carried out

on the monkey has usually been hidden in reports on other animal forms. Kahn (1912) kept two *macacus rhesus* monkeys alive each for 5 days after adrenal removal, and in gross determinations found only traces of glycogen present in the liver after adrenal insufficiency had set in. Apparently there is some difficulty in maintaining adrenalectomized monkeys alive with cortico-adrenal extract. Hartman and Winter (1933) have reported a series of four animals (macaques) which lived 4, 16, 26 and 35 days after operation with the aid of "cortin." Activity and infection were found to bring about marked exhaustion from which resuscitation could not be effected even with large doses of extract. Harrop and Weinstein (1933) kept two macaques alive on extract 11 and 42 days after adrenalectomy, and noted skin pigmentation in the longer-lived case. Firor and Grollman (1933) state that they made observations on 6 monkeys, but give no survival periods or other data.

Allen and his colleagues (1937) kept two adrenalectomized-ovariectomized monkeys alive for short periods with cortico-adrenal extract. One partially adrenalectomized macaque survived for a few weeks and another for a few months in Stewart and Rogoff's laboratory (1919).

No quantitative blood-chemical or tissue analyses were mentioned in the above brief reports, and only a few individuals were considered. The present work covers in monkeys the more important changes such as are known to occur in adrenal insufficiency in lower animal types. It is apparent that blood and tissues are affected similarly as far as direction of change is concerned. All the New-World monkeys examined, however, appear to suffer much more profoundly than other animals from carbohydrate deficiencies following adrenal removal. Almost certainly the figures for liver glycogen and serum sugar indicate complete exhaustion in these depots. They are in sharp contrast to the high levels found in the unoperated animals. It should be emphasized, too, that food was taken fairly readily by the monkey when prostrated from adrenal insufficiency, and food residues were usually found in the gastro-intestinal tract at autopsy. Besides the liver and blood changes, muscle and heart glycogen reductions were also severe.

The hypoglycemic convulsions, which persisted for several hours preceding death from adrenal loss, were in correlation with the carbohydrate losses. We have studied to date many animal series covering more than a dozen different mammalian species suffering from adrenal insufficiency, and in no other animal have the convulsive seizures been so severe and prolonged, and the carbohydrate reserves so low, as in the present series of monkeys. It is clear that these primates from which the adrenals have been removed suffer most and die chiefly from carbohydrate deficiencies.

In a recent paper Grollman (1938) reports that the cortico-adrenal hormone is well able to maintain normal carbohydrate levels in adrenal-

ectomized or adrenalectomized-pancreatectomized rats. He was unable to influence blood sugar in normal and in hypophysectomized rats with cortico-adrenal extracts, however, and hence did not consider that the adrenal cortex was preëminently involved in regulating carbohydrate metabolism. Grollman, it should be emphasized, used only very small amounts of extract—1 cc. or less—in rats which are notoriously resistant to hormones. In earlier papers we have already demonstrated the positive action of our adrenal extracts on carbohydrate levels, however, in both normal and hypophysectomized animals (Britton and Silvette, 1932; Corey, 1938). The present and other recent experiments (Britton and Silvette, 1937; Britton, Silvette and Kline, 1938) have further shown the revolutionary effects of adrenal removal on carbohydrate metabolism in a wide variety of animal types.

SUMMARY

New-World monkeys—capuchins, night-monkeys, marmosets—show tissue and blood chemical changes after adrenal removal which are rather similar to those found in other higher mammalian forms. Serum sodium and chlorides are significantly reduced while muscle water tends to be increased. Serum potassium and urea are raised.

Extreme carbohydrate changes, more profound than those which occur in other mammalian types, were observed. In 9 cases serum glucose reductions in adrenal insufficiency averaged over 70 per cent, and in 12 cases liver glycogen values were reduced over 90 per cent (table 1). These figures appear to represent exhaustion of available carbohydrates in these depots. In muscle and cardiac tissues glycogen levels were also significantly reduced.

The very low carbohydrate levels in adrenal insufficiency contrast with markedly high liver glycogen values, which may approximate 12 per cent, found in normal monkeys.

Hypoglycemic convulsions are a prominent feature of adrenal insufficiency in monkeys. Usually they last over a period of several hours and are characterized by very severe spasms, eventually giving place at the last to seizures of an asphyxial type.

Evidently the highly important function of the liver as a former and furnisher of glycogen is rapidly abolished in the absence of the cortico-adrenal hormone. Further, there appears no doubt that the first cause of death in adrenal insufficiency in the primates examined is the exhaustion of carbohydrates in blood and hepatic tissues.

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THE NORMAL ANTITHROMBIN OF THE BLOOD AND ITS RELATION TO HEPARIN[1]

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Received for publication June 2, 1938

In 1936 the writer (1) corroborated the finding of Howell (2) that in peptone shock the blood contains a substance similar to or identical with heparin, and concluded that the incoagulability of the blood was due to this antithrombic agent. A year later Eagle, Johnston and Ravdin (3) reported that the prolonged coagulation time in anaphylactic shock likewise was caused by an antithrombin. These findings again direct attention to the importance of antithrombins in the coagulation of the blood. Much confusion, however, still exists in the interpretation of the nature and action of antithrombic agents. Eagle and his associates believe that in anaphylactic shock, the normal antithrombin of the blood is merely greatly increased. The writer (4), on the contrary, has presented evidence that the normal antithrombin of the blood is serum albumin or a substance closely associated with this protein, and that in shock a new type of antithrombin appears, which is not demonstrable in normal blood, and that the action of the new substance is very similar to that obtained when heparin is added to plasma.

Obviously, one of the most important questions to be answered before the present confusion concerning antithrombins can be clarified is what substance in the blood constitutes the normal antithrombin. It is rather surprising that so little attention has been paid to the serum proteins as the most likely substances responsible for the neutralization of thrombin. Rettger (5) in 1909 clearly stated his belief that thrombin unites with the blood proteins, and four years later Landsberg (6) observed that thrombin could combine with serum albumin. Little consideration was given to the possible significance of these observations, and subsequent workers usually contented themselves with creating hypothetical antithrombins whenever needed.

In the present paper evidence is presented which strongly supports the idea that the antithrombic action of the blood is due mainly to serum al-

[1] This paper was presented before the Forty-ninth Meeting of the American Physiological Society, at Memphis, April, 1937.

bumin. By means of simple but essentially quantitative methods it has been possible to demonstrate that a solution of serum albumin exhibits an antithrombic action remarkably similar to serum itself. It seems fairly certain that serum albumin is not a single substance and therefore it is probably more correct to say that perhaps a constituent of the albumin fraction is responsible for the antithrombic action. Whenever the term albumin is used in this paper, this possibility is implied.

EXPERIMENTAL. *Preparation of thrombin.* By utilizing the finding of Addis (7) and Eagle (8) that a portion of the prothrombin is precipitated when carbon dioxide is passed through a cold and greatly diluted plasma, a very active solution of thrombin can be prepared (9). With fresh human plasma, a preparation of thrombin can be made which is so constant in potency that it can be employed as a standard. In the present study, this concentrated solution of thrombin is designated as full strength (F. S.). When 0.2 cc. of rabbit or human plasma is mixed with 0.1 cc. of the standard thrombin solution, coagulation occurs in 3 seconds. With progressive dilutions of the thrombin solution, the clotting time becomes correspondingly longer:

Dilution of thrombin	F.S.	1/2	1/5	1/10	1/20	1/40	1/100	1/200	1/300
Clotting time in seconds	3	4	7	11	18	33	80	180	240

It can readily be seen that the clotting time can be used as a direct measure of the thrombin content of a solution, and also for determining the rate of disappearance of thrombin from a mixture.

Heparin. The studies on heparin were conducted with a preparation made according to the method of Charles and Scott (10), but the results were checked with a highly purified heparin kindly supplied by Doctor Best. No difference in action between the two preparations could be found except in potency.

The neutralization of thrombin by plasma. By mixing thrombin with plasma, and then testing the coagulating power of the mixture on plasma, a rapid diminution of thrombic activity occurs as seen in table 1. The speed with which thrombin is neutralized is accelerated as the amount of plasma is increased.

The thermolability of the antithrombic substance in plasma, which was clearly recognized by Howell (11) and others, can be readily demonstrated. Serum or plasma heated for 15 minutes at 66–67°C. almost completely loses its antithrombic action as shown in table 2.

These simple experiments suggest that the antithrombic activity must be attributed to a substance which is altered by heat, and which is present in the blood in a relatively high concentration. Of the known substances

in serum, the proteins definitely fulfill these requirements. It remains to be determined which of two proteins, the globulin or the albumin, possesses this strong antithrombic action.

The antithrombic action of serum globulin and albumin. For the separation of globulin from albumin Howe's method was employed: 20 cc. of

TABLE 1

*The neutralization of thrombin by plasma**

Plasma ..... 0.2 cc. Thrombin (F. S.)..... 0.8 cc.	Plasma ..... 0.4 cc. Thrombin (F. S.)..... 0.8 cc.	Plasma .....0.8 cc. Thrombin (F. S.).....0.8 cc.
After incubating	After incubating	After incubating
1 min., clot† in 5 sec.	1 min., clot in 5.5 sec.	1 min., clot in 9.5 sec.
3 min., clot in 8 sec.	3 min., clot in 13 sec.	3 min., clot in 40 sec.
5 min., clot in 13 sec.	5 min., clot in 21 sec.	5 min., clot in 85 sec.
10 min., clot in 21 sec.	10 min., clot in 42 sec.	10 min., clot in 195 sec.
15 min., clot in 31 sec.	15 min., clot in 80 sec.	15 min., clot in 600 sec.
20 min., clot in 43 sec.	20 min., clot in 105 sec.	
25 min., clot in 50 sec.	25 min., clot in 180 sec.	

* The fibrinogen was removed by adding 0.2 cc. of thrombin (F.S.) to 5 cc. of rabbit plasma.

† The clotting time was determined by adding 0.1 cc. of the incubated mixture to 0.2 cc. of rabbit plasma. The same procedure was employed in tables 2, 3, and 4.

TABLE 2

The thermolability of the antithrombic substance in plasma

Heated plasma* 0.5 cc. Thrombin (F.S.).....0.5 cc.	Unheated plasma.....0.5 cc. Thrombin (F.S.).....0.5 cc.
After incubating	After incubating
1 min., clot in 5 sec.	1 min., clot in 10 sec.
3 min., clot in 5 sec.	3 min., clot in 33 sec.
5 min., clot in 6 sec.	5 min., clot in 90 sec.
10 min., clot in 6.5 sec.	10 min., clot in 200 sec.
20 min., clot in 8 sec.	15 min., clot in 600 sec.
60 min., clot in 11 sec.	

* Heated at 66 to 67°C. for 15 minutes.

TABLE 3

A comparison of the antithrombic action of serum globulin and albumin

Albumin solution*.....0.5 cc. Thrombin (F.S.)..... 0.5 cc.	Globulin solution†.....0.5 cc. Thrombin (F.S.).....0.5 cc.
After incubating	After incubating
0 min., clot in 6 sec.	0 min., clot in 6 sec.
3 min., clot in 60 sec.	3 min., clot in 7 sec.
5 min., clot in 180 sec.	5 min., clot in 7.5 sec.
10 min., clot in 600 sec.	10 min., clot in 9 sec.
	20 min., clot in 12 sec.
	30 min., clot in 15 sec.

* The solution contained approximately as much albumin as the original plasma (3.25 grams per 100 cc.).

† The solution contained approximately 4 times as much globulin as the original plasma.

oxalated rabbit plasma (from which the fibrinogen had been removed through coagulation by means of adding 0.2 cc. of the concentrated thrombin solution) was mixed with 600 cc. of 22.2 per cent sodium sulfate solution and allowed to stand one hour. The precipitated globulin was filtered off, redissolved in 10 cc. of physiological saline solution, and again

precipitated with 100 cc. of 22.2 per cent sodium sulfate. The precipitate was dissolved in 5 cc. of normal saline solution and dialyzed for 2 hours against saline solution using a modified Simms concentrating dialyzer. The final volume of the globulin solution was 5 cc. and contained roughly 4 times as much globulin as serum.

The clear filtrate obtained after the removal of the globulin was warmed to 40°C. and 150 grams of powdered ammonium sulfate added. The precipitated albumin was dissolved in 20 cc. of distilled water, and dialyzed against normal saline solution for 2 hours. The final volume of the solution was 20 cc. which approximated the concentration of albumin in the original plasma. To test the antithrombic action of the globulin and albumin solutions, a definite volume was mixed with a known amount of the standard thrombin solution. To determine the disappearance of thrombin, 0.1 cc. was removed at definite time intervals, added to 0.2 cc. of plasma and the coagulation time observed. The results which are recorded in table 3 clearly show that globulin has a very weak antithrombic action in comparison to the strong thrombin neutralizing power of albumin. The rate at which thrombin is neutralized by albumin depends on the concentration of the latter. A comparison of the speeds with which serum and albumin inactivate thrombin indicates that the antithrombic activity of the former is dependent on its albumin concentration.

Since the antithrombic power of serum is almost completely destroyed on heating to 67°C., it is to be expected that albumin likewise would lose its antithrombic action at that temperature. This is demonstrated in table 4. It should be noted that the power of the serum or albumin to neutralize thrombin is nearly completely destroyed at 67°C.; nevertheless a slight antithrombic activity remains. At this critical temperature profound changes in the albumin begin to occur and at a temperature a degree or two higher, turbidity sets in, and at 70°C. flocculation occurs. The labile character of the antithrombic property is also shown by the fact that a marked diminution of the thrombin neutralizing power occurs when an albumin solution is allowed to stand a few days in a refrigerator.

It seems quite evident that the antithrombic action of albumin is specific, and cannot be satisfactorily explained on the basis of adsorption. It has been found that fresh egg albumin has no antithrombic action, a finding entirely in accord with the observation of Gasser (12), who reported that fresh whole milk, peptone, potato starch, acacia, gelatin, animal charcoal and kaolin likewise do not act antithrombically.

While the existence of an antithrombin in the blood is widely accepted, there is no general agreement concerning its relation to the normal coagulation process. It can be calculated from the data in table 1 that when 0.1 cc. of plasma is mixed with 0.4 cc. of thrombin solution, over 80 per

cent of the thrombic activity is destroyed in 5 minutes; or in other words 0.1 cc. of plasma can neutralize 0.3 cc. of thrombin in that period of time. Yet, if 0.2 cc. of plasma is mixed with 0.1 cc. of thrombin diluted 1 to 300, a slight but definite clot will appear in about 3 to 4 minutes, and a solid clot in about 30 minutes. Thus 0.2 cc. of plasma will be coagulated by 0.1 cc. $\times$ 1/300 or 0.0003 cc. of thrombin (F. S.), yet 0.1 cc. of defibrinated plasma can inactivate 0.3 cc. of thrombin (F. S.) in 5 minutes. From these results it can readily be calculated that plasma is clotted by an amount of thrombin which is only 1/2000 of the quantity which the defibrinated plasma can inactivate in 5 minutes. The answer to this apparently paradoxical observation was given many years ago by Gasser (12) when he wrote: "In normal coagulation the thrombin formed has two possible fates. It may be combined with antithrombin or with fibrinogen. The division coefficient between these two would be a valuable bit of information."

Although the division coefficient cannot be expressed as a mathematical value, it seems evident that the affinity of thrombin is so much greater for fibrinogen than for albumin, that very little thrombin is neutralized as long as fibrinogen remains. The fact that a dilute solution of thrombin will gradually coagulate the fibrinogen of a relatively large amount of plasma clearly suggests that thrombin unites with fibrinogen, is reliberated after fibrin is formed, and so continues until all the fibrinogen is coagulated. While the coagulation of 0.2 cc. of plasma by 0.1 cc. of a 1 to 300 dilution of thrombin, is surprising, even more striking results would undoubtedly be obtained were it not for the fact that thrombin is gradually lost by adsorption to fibrin. The behavior of thrombin in plasma can be expressed as follows:

Fibrinogen + thrombin = Fibrinogen—thrombin (an intermediary compound) = Fibrin + thrombin.

After all the fibrinogen is coagulated: Albumin + thrombin = Albumin—thrombin (metathrombin).

In the clotting of blood in a test tube, it is fairly certain that at the moment of coagulation more thrombin is formed than can combine with fibrinogen and that the excess is immediately neutralized by the albumin or a component in the albumin fraction. It seems improbable, however, that any increase of this normal antithrombin factor could exert any significant inhibitory action on coagulation.

One must carefully differentiate between an antithrombic agent and an anticoagulant. A substance which binds or neutralizes thrombin is an antithrombin, but it only becomes an anticoagulant when its affinity for thrombin exceeds that of fibrinogen. Serum albumin, or a substance closely associated with it, is distinctly an antithrombin but not an anticoagulant.

Heparin, an antithrombogen. Before considering the experiments designated to demonstrate the true anticoagulant action of heparin, it should be mentioned that the author (13) has shown that this agent does not prevent the conversion of prothrombin to thrombin, and furthermore that it is not neutralized by thromboplastin (9). In complete agreement with Howell's (2) early finding, the writer has previously shown that when heparin is tested against pure fibrinogen and thrombin, it exhibits little antithrombic power, whereas in plasma it becomes one of the most powerful antithrombic substances known. This indicates that the antithrombic action results from the reaction of heparin with a constituent of the plasma. Howell came to this conclusion early in his work on heparin, and recently this view has again been expressed by Schmitz and Kühl (14).

It can be shown that the strong antithrombic action of plasma containing heparin is not due to the simple addition of the antithrombic power of heparin and the normal antithrombin of the plasma. If 0.5 cc. of thrombin (F. S.) are added to 0.5 cc. of plasma containing 0.3 mgm. of heparin, no coagulation occurs. In other words, 0.6 mgm. of heparin in 1 cc. of plasma will instantly inactivate 1 cc. of thrombin (F. S.). To show that this value is not the simple sum of the antithrombic power of plasma and heparin, one merely has to determine the antithrombic value of these two separately. It will be recalled that 0.1 cc. of a 1 to 300 dilution of thrombin will coagulate 0.2 cc. of plasma, or 0.5 cc. of a 1 to 300 dilution of thrombin which is equivalent to 0.0017 cc. of thrombin (F. S.), can coagulate 1 cc. of plasma. The anticoagulating power of 1 cc. of plasma must therefore be less than 0.0017 cc. of thrombin (F. S.). To determine the intrinsic power of heparin to neutralize thrombin, 50 mgm. of heparin was added to 1 cc. of thrombin diluted 1 to 10. After a 10 minute incubation, 0.1 cc. of this mixture was added to 0.2 cc. of fibrinogen. Coagulation occurred in 40 seconds. Since 50 mgm. of heparin did not neutralize 1 cc. of thrombin diluted 1 to 10, it can be concluded that to neutralize 1 cc. of thrombin (F. S.) would require more than 500 mgm. of heparin. Therefore, 0.6 mgm. of heparin will neutralize less than the 6/5000 of 1 cc. of thrombin (F. S.) or 0.0012 cc.

While 0.6 mgm. of heparin when added to plasma will neutralize 1 cc. of thrombin (F. S.) the antithrombin sum of the plasma and heparin separately is less than 0.0017 cc. + 0.0012 cc. or 0.0029 cc. Thus, the antithrombic activity produced by adding heparin to plasma is over 300 times greater than could be expected were it a simple addition of the intrinsic antithrombic power of heparin to the normal antithrombin of the plasma. It is very evident that heparin in plasma gives rise to a new and powerful antithrombin.

Immediately the question arises which substance in plasma reacts with heparin to form this new anticoagulant. Again the serum proteins seemed

the most logical substances to be considered, and readily furnished the answer as can plainly be seen in table 5. A minute amount of heparin when allowed to react with albumin or a substance present in serum albumin, produces a powerful inactivator of thrombin. In contrast, no anticoagulant is formed when heparin is added to globulin. These observations indicate that the antithrombic power of heparin is dependent upon

TABLE 4

The destruction of the antithrombic power of albumin by heat

Heated albumin solution.....0.5 cc. Thrombin (F.S.).0.5 cc.	Unheated albumin solution.....0.5 cc. Thrombin (F.S.)..0.5 cc.
After incubating 0 min., clot in 6 sec. 3 min., clot in 10 sec. 5 min., clot in 11 sec. 10 min., clot in 15 sec. 20 min., clot in 25 sec.	After incubating 0 min., clot in 6 sec. 3 min., clot in 60 sec. 5 min., clot in 180 sec.

The albumin solution was heated for 5 minutes at 64°C. No turbidity was observed. In another solution of albumin, incipient flocculation was observed on heating it to 70°C., and when mixed with an equal volume of thrombin (F.S.) and incubated for 1 hour still clotted oxalated plasma in 7 seconds.

TABLE 5

A comparison of the effectiveness of heparin to produce antithrombin in serum albumin and globulin

Globulin solution.....0.5 cc. Heparin.....0.5 mgm. Thrombin (F.S.).....0.5 cc.	Albumin solution.0.5 cc. Heparin..0.06 mgm. Thrombin (F.S.)...0.5 cc.
After incubating 1 min., clot in 13 sec. 3 min., clot in 17 sec. 5 min., clot in 21 sec.	Tested immediately. No clot

The clotting time was determined by adding 0.1 cc. of the incubated mixture to 0.2 cc. of a fibrinogen solution.

TABLE 6

The ineffectiveness of heparin to produce an antithrombic action in plasma or in an albumin solution which has been heated

Heated plasma*.....0.5 cc. Heparin.....5.0 mgm. Thrombin (F. S.).....0.5 cc.	Heated albumin sol.*.....0.5 cc. Heparin.....1.0 mgm. Thrombin (F. S.).....0.5 cc.	Unheated plasma.....0.5 cc. Heparin.....0.3 mgm. Thrombin (F.S.).0.5 cc.
After incubating 1 min., clot in 11 sec. 30 min., clot in 13 sec.	After incubating 1 min., clot in 13 sec.	Tested immediately No clot

In each case 0.1 cc. of the mixture was added to 0.2 cc. of fibrinogen solution.
* Heated for 15 min. at 67°C.

serum albumin. This conclusion is further supported by the fact that if albumin is heated to 67°C., the addition of even large amounts of heparin will no longer bring about the production of an anticoagulant (table 6). Likewise when plasma is heated, it also loses its power to react with heparin to form the powerful antithrombin present in unheated plasma to which heparin has been added.

A logical explanation for these findings can be offered. Albumin has a

specific ability to unite with thrombin, but fibrinogen has a greater affinity. Coagulation therefore is not inhibited by albumin and little thrombin is inactivated before all the fibrinogen is converted to fibrin. On adding heparin to albumin, a reaction apparently occurs which so increases the affinity of albumin for thrombin that it exceeds that of fibrinogen; consequently the thrombin is combined with the heparinized albumin and inactivated before it can unite and react with fibrinogen. Heparin converts albumin from a relatively weak antithrombin to a powerful anticoagulant. When albumin is heated, it is altered chemically and loses its property to combine with thrombin. When this occurs, the addition of heparin is ineffective since the latter can merely intensify the intrinsic antithrombic action of albumin, but cannot restore it. The nature of the reaction between heparin and albumin remains to be determined, but it is interesting that Schmitz and Fischer (15) found that heparin shifted the isoelectric point of serum albumin to the acid side.

SUMMARY

The experiments presented suggest that the normal antithrombin of the blood is albumin or a constituent closely associated with that fraction. Since fibrinogen has a greater affinity for thrombin than albumin, no appreciable neutralization or inactivation of thrombin occurs until all of the fibrinogen is converted to fibrin. By adding heparin to albumin, the affinity of the latter for thrombin is so increased that the thrombin will be combined with the heparin-albumin complex before it can react with fibrinogen. When albumin is heated to 67°C., it loses its ability to neutralize thrombin, and the addition of heparin no longer produces any antithrombic action, which indicates that heparin merely intensifies the inherent antithrombic power of albumin and is itself not an antithrombin.

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RENAL CLEARANCES OF IOPAX, NEOIOPAX AND SKIODAN IN MAN

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Received for publication June 6, 1938

The organic iodine compounds, diodrast and hippuran, have the highest clearances in the human kidney of any known substance, these clearances being ten times greater than the urea clearance, five to six times greater than the inulin clearance (2, 3). Considerable interest attaches to the fact that synthetic compounds which are entirely foreign to the body should be cleared from the blood so much more effectively than is urea, the chief nitrogenous end-product of metabolism. In view of the fact that these two substances have little in common in their chemical structure beyond the presence of firmly bound iodine, it seemed of interest to examine the renal clearances of other organic iodine compounds, iopax (uroselectan), neoiopax and skiodan. It has been shown elsewhere (4) that all these compounds are bound to some extent to plasma proteins and are therefore not completely filtrable at the glomerulus. The methods used here are those reported by Smith, Goldring and Chasis (3). The subjects were normal, volunteer patients. Inulin and phenol red were given by constant intravenous infusion, the latter being present in such concentrations as to maintain a plasma level slightly below 1 mgm. per cent. The clearances of the iodine compounds were examined either at low, constant plasma concentrations, or on a rising plasma curve obtained by following the protocol of figure 3 of Smith, Goldring and Chasis. The absolute quantities of the iodine compound in the infusion fluid, which need not be detailed, must be adjusted to the value of the clearance.

Iopax. The excretion of iopax has been examined in two subjects. In one subject (fig. 1) the iopax/inulin clearance ratio at plasma concentrations of 3.0 to 4.4 mgm. per cent of iodine averaged 4.26. As the concentration of iopax was elevated in the blood the iopax clearance was depressed, the iopax/inulin clearance ratio falling to 1.48. The lowest curve, *F*, in figure 1 indicates the fraction of iopax in the plasma which is free and therefore filterable through the glomeruli, as calculated from the albumin content of the plasma using the data on protein binding which have been reported elsewhere (4). Were there no tubular excretion

at the concentrations examined, the iopax/inulin clearance ratio should be identical with this curve. The fact that the clearance ratio is greater than the expected value shows that this substance is copiously excreted by the tubules. The reason that the iopax clearance is depressed at elevated plasma levels is presumably due to a limitation in the rate of tubular excretion, as in the case of diodrast, hippuran, and phenol red. An apparent maximal rate of tubular excretion, calculated according to Smith, Goldring and Chasis, appears to have been reached in this subject at a plasma level of 11 mgm. per cent of iodine, and to have a value of 27 mgm. of iodine (0.21 mM) per minute.

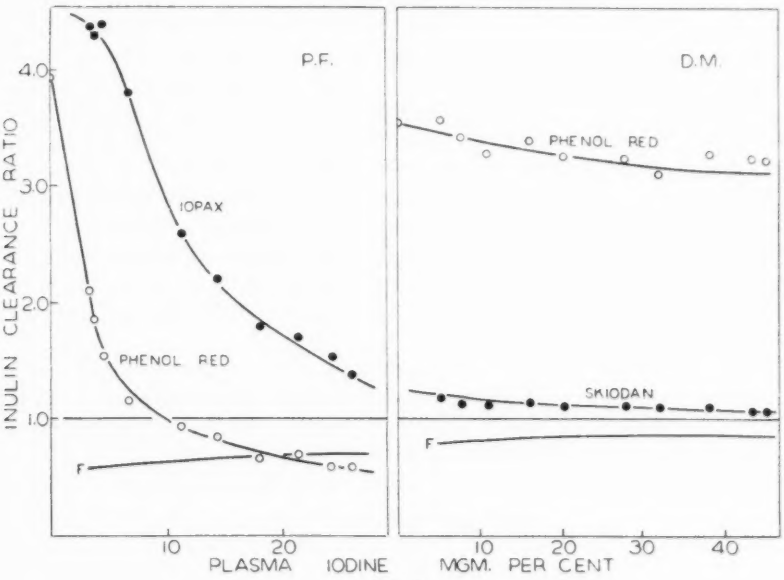

Fig. 1

Diodrast and hippuran markedly depress the simultaneous phenol red clearance, a fact which has been interpreted as indicating that these substances are excreted by a common mechanism in the renal tubules (3). As shown in figure 1, iopax has a similar action in depressing the simultaneous phenol red clearance. As in the case of diodrast and hippuran, this depressive action is greater upon the phenol red clearance than upon its own clearance. Simultaneous clearances of phenol red and iopax at low plasma levels of the latter were not examined in this subject.

In subject S. M. the iopax/inulin clearance ratio averaged 5.7 and the phenol red/inulin clearance ratio averaged 2.5, at a plasma level of 0.64 to 0.70 mgm. per cent iopax iodine; these ratios were depressed, in a manner

comparable to that shown in figure 1, to 1.33 and 0.46, respectively, at 26.0 mgm. per cent iodine. The phenol red/iopax clearance ratio averaged 0.44 at the low plasma levels of iopax. The maximal rate of tubular excretion was reached at approximately 10 mgm. per cent of iodine and averaged 21 mgm. of iodine (0.17 mM) per minute.

Our observations are too limited in number to assert definitely that the iopax clearance is less than the hippuran clearance. In the absence of analytical methods permitting the measurement of simultaneous clearances of the two compounds, a decision in this matter would require a statistically valid series of independent comparisons.

Skiodan. Elsom, Bott and Shiels (1) have shown that the skiodan clearance in the dog averages about 93 per cent of the simultaneous creatinine clearance (rate of filtration), and that the ratio between these clearances is independent of the concentration of skiodan in the plasma. If the fraction of free skiodan in dog plasma is of the same order of magnitude as in human plasma (4), from 92 to 93 per cent would be free and therefore filterable at the concentrations examined by these investigators, and this circumstance would account for the observed skiodan/creatinine clearance ratio without invoking tubular reabsorption or excretion.

Landis, Elsom, Bott and Shiels (2) have examined the excretion of skiodan in man, but report only four clearance periods in two subjects. The skiodan/creatinine clearance ratio ranged from 0.82 to 1.14 and averaged 0.95.

The excretion of skiodan has been examined by us in two subjects. In both, the skiodan clearance at plasma levels of 4 to 6 mgm. per cent of skiodan iodine was about 20 per cent above the inulin clearance; on elevation of the skiodan plasma level to 36.5 and 45.2 mgm. per cent iodine the skiodan clearance was slightly depressed, but did not fall below the inulin clearance. The data on one subject are given in figure 1. The curve, F, shows the fraction of skiodan filterable from mixed human plasma at the observed concentrations. We believe that the discrepancy between the expected and observed skiodan/inulin clearance ratios is sufficiently great to justify the conclusion that this substance is excreted by the tubules in man to a very slight extent. This conclusion is supported by the fact that in both subjects elevation of the skiodan plasma level depressed the simultaneous phenol red/inulin clearance ratio to a significant degree. A maximal rate of tubular excretion was not reached in either subject; if such exists, its value lies above 14 mgm. of skiodan iodine (>0.1 mM) per minute. It is interesting to note that Elsom, Bott and Shiels report that skiodan is excreted by the aglomerular fish kidney, an observation in line with the present evidence that it is excreted by the renal tubules in man.

Neoiopax. We have examined the excretion of neoiopax in two subjects. In one, the neoiopax/inulin clearance ratio at plasma levels of

neoiopax iodine of 1.0 mgm. per cent averaged 1.24; this ratio was depressed to 0.99 at 12 mgm. per cent of neoiopax iodine. In the second subject the clearance ratio averaged 1.15 at plasma levels of 5 mgm. per cent of iodine and was depressed to 0.80 at a plasma level of 41 mgm. per cent iodine. The calculated filterable fraction of neoiopax ranges from 0.72 to 0.89 at these plasma levels. The discrepancy between these figures and the observed neoiopax/inulin clearance ratios indicates that this substance is also excreted by the renal tubules. This conclusion is fortified by the fact that elevation of the plasma level of neoiopax markedly de-

TABLE 1

		AVERAGE INULIN CLEARANCE RATIO	T_m* mM. PER MINUTE	PER CENT FREE†	APPROXIMATE PER CENT DEPRESSION EFFECTED BY 1.0 mM. PER LITER IN PLASMA	
					On self clearance	On simultaneous phenol red clearance
Diodrast	3,5-*di*iodo-4-pyridone-N-acetic acid	6.0	0.3	72	50	85
Hippuran	Sodium ortho-iodohippurate	5.4	0.6	30	25	80
Iopax	Sodium 2-oxo-5-iodopyridine-N-acetate	5.0	0.2	56	65	75
Neoiopax	Disodium N-methyl-3:5-*di*iodo-4-pyridoxyl-2:6-dicarboxylate	1.2	0.01	74	25	55
Skiodan	Sodium mono-iodomethane sulphonate	1.2	>0.1	82	±5	±5

* The maximal rate of tubular excretion. The data on diodrast and hippuran are quoted from Smith, Goldring and Chasis (3).

† Per cent of total dye filterable at a total concentration of 1.0 mgm. per cent. in human plasma containing 4 per cent albumin.

presses the phenol red/inulin clearance ratio. In the first subject the phenol red/inulin clearance ratio was depressed at 12 mgm. per cent of iodine to 86 per cent of its control value; in the second subject this ratio was depressed at 41 mgm. per cent of iodine to 46 per cent of its control value.

There appears to be a maximal rate of tubular excretion of neoiopax of the order of magnitude of 3 mgm. of iodine (0.01 mM.) per minute, but this figure is subject to a large error by the nature of its determination.

There is no evident relationship between tubular excretion and chemical

structure among these organic compounds, as is shown by the data in table 1, except for the pyridine nucleus in diodrast, iopax and neoiopax, and for the fact that they all contain firmly bound iodine. But there are certain general relations between the simultaneous clearances which are of interest. Smith, Goldring and Chasis have shown that diodrast and hippuran, which have higher clearances than phenol red, depress the simultaneous phenol red clearance more than they depress their own clearances. On the other hand, phenol red has only a slight action in depressing the diodrast clearance. They interpret this to indicate a quantitative competition between the two solutes for the excretory mechanism of the renal tubules. A more precise analysis of the quantitative relations involved here would require the observation of simultaneous clearances at constant or known renal blood flows, preferably in one subject, and it should include an examination of the action of one iodine compound upon the clearance of another. But our present data permit the extension of the quantitative principle to the extent of demonstrating that the greater the inulin clearance ratio—i.e., the greater the affinity, so to speak, of the iodine compound for the excretory mechanism—the greater is the tendency of the compound to displace phenol red from the mechanism and to prevent its excretion. Whether this principle of competition extends to all solutes excreted by the renal tubules, or is confined to certain groups, is undetermined.

SUMMARY

Evidence is presented that the organic iodine compounds, iopax, neoiopax, and skiodan, are excreted in part by the renal tubules in the human kidney. As in the case of other substances excreted by tubular activity, the clearances are depressed as the plasma concentration is elevated. All these substances, when present in increasing plasma concentrations, progressively depress the phenol red clearance. It appears that the greater the tubular clearance of the organic iodine compound, the greater is the capacity to depress the phenol red clearance. This result is consonant with the belief that these substances are all excreted by a common cellular mechanism, and that they enter into quantitative competition for that mechanism.

We are indebted to Prof. H. W. Smith for assistance in the preparation of this paper.

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THE EFFECT OF BILATERAL LIGATION OF THE LUMBO-ADRENAL VEINS ON THE COURSE OF PANCREAS DIABETES[1]

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Received for publication June 6, 1938

The importance of the endocrine balance for carbohydrate metabolism has been emphasized by Houssay's work (1) which discloses that hypophysectomy of the dog and toad relieves the symptoms of experimental diabetes. Long (2) has demonstrated that adrenalectomy combined with injections of cortin results in a similar ameliorative action in depancreatized cats and dogs. Grollman and Firor (3) have emphasized the interrelationship of the pituitary and the adrenal cortex. With chronic insufficiency produced by bilateral ligation of the venous return from the adrenal glands, a syndrome develops which they interpret as being partly of pituitary origin. In the present experiments an attempt was made to determine the effect of ligation of the lumbo-adrenal veins on experimental diabetes.

METHOD. Under pentobarbital anesthesia the lumbo-adrenal veins of cats and dogs were ligated proximally and distally to the gland and the pancreas was removed at the same time. After recovery from the operation the animals were placed on various diets (bovex, minced beef, glucose) but received neither insulin nor cortin. No sodium chloride or choline was added to the dietary. Observations were made of the survival period, urinary glucose, nitrogen and ketonuria, and blood glucose, fat and oxygen capacity as well as of the total base of the serum. The carbohydrate balance and the respiratory metabolism were also determined. In a few instances analyses were made of liver and kidney for fat.

RESULTS. 1. *Survival.* Thirteen dogs subjected to the combined operation survived for a period ranging from 8 to 33 days with an average of 17.5 days. Similarly 20 cats lived for 8 to 98 days after operation. Omitting the one cat which lived for 98 days, the average was 19 days; the survival period varied from 8 to 31 days. This series does not include 12 cats and 5 dogs that lived from 4 to 7 days postoperative nor the animals which died within the first 3 days after the operation.

[1] Aided by grant from the National Research Council.

Biochemical studies. The average glucose, nitrogen and ketone substances per kilogram per day and the D/N of the dogs are presented in table 1. This table also contains average values observed on cats receiving 100 grams minced beef daily.

Blood constituents. Because of the variability of the blood picture average values would not be representative. For this reason the results on 4 dogs are presented in table 2: dogs 1 and 11 with prolonged survival despite a hyperglycemia and dog 15 whose blood constituents could not be differentiated from those of normal unoperated animals. Dog 7 died with hypoglycemia.

In an attempt to evaluate the degree of adrenal insufficiency in our animals, studies of the serum total base were made on various occasions during the survival period including the time just previous to the sacrifice of the animal. In only 2 of the 7 animals was the premortal total base

TABLE 1

Urinary constituents of depancreatized dogs and cats with bilateral ligation of the lumbo-adrenal veins

	DEXTROSE	NITROGEN	D/N	KETONE	
	gm./kgm./day	*gm./kgm./day*		*mgm./kgm./day*	
Dogs fasted	0.83	0.54	1.36		Average
	0–1.49	0.33–0.74	0–1.63		Range
Dogs fed	0.71	0.59	0.90	22	Average
	0–2.15	0.32–0.90	0–2.39	7.5–27	Range
Cats fed	1.18	0.76	1.56		Average
	0.358–1.71	0.343–1.06	0.46–3.35	Trace	Range

significantly decreased below normal (139 milliequiv. and 125 milliequiv.), the other observations being 155, 171, 146, 153, 143 milliequiv.

In most instances blood fat was slightly raised. Twenty-seven observations of blood fat of dogs during various periods of survival yielded an average of 396 mgm. per cent, the range being from 243 to 560 mgm. per cent.

Tissue fats. An analysis of the liver and kidney tissue fat of 8 cats revealed that at the time of sacrifice the liver fat ranged from 4.2 to 6.0 per cent, a slight increase above the normal amount. Kidney fat in contrast was markedly elevated, ranging from 6.4 to 14.8 per cent.

Respiratory metabolism. Twenty-eight observations of the respiratory metabolism were made on 6 cats yielding an average total R.Q. of 0.786 and 41 observations on 10 dogs disclosing an average R.Q. of 0.792. Six observations were made on cats and 8 on dogs before and after the inges-

tion of glucose. In only 2 instances with the cats was there a significant rise in R.Q. No increase after the administration of glucose was observed in the dogs.

Carbohydrate balance. Carbohydrate balance studies were conducted on 10 dogs. From 12 to 25 grams of glucose were given to each animal for several consecutive days. It was observed that regardless of the level of the blood sugar these animals were able to retain varying amounts

TABLE 2

Blood studies of depancreatized dogs with bilateral ligation of the lumbo-adrenal veins

DOG NO.	DATE	BLOOD GLUCOSE	SERUM, TOTAL FATTY ACIDS	SERUM, TOTAL BASE	SURVIVAL PERIOD
		mgm. per cent	*mgm. per cent*	*milli-equivalent*	*days*
1	10/22/37 operated				33
	10/28/37	241	397	153	
	11/ 1/37	262	359	169	
	11/ 5/37	260	274	147	
	11/15/37	348	318	165	
	11/22/37	344	336		
	11/21/37 sacrificed				
11	1/10/38 operated				31
	1/17/38	241	391	169	
	1/31/38	284	512	139	
	2/10/38 sacrificed				
7	12/22/37 operated				18
	1/ 3/38	280	360		
	1/ 5/38	317	354		
	1/10/38	30	243	171	
	1/10/38 sacrificed				
15	1/10/38 operated				21
	1/17/38	80	285	159	
	1/31/38	75	353	153	
	1/31/38 sacrificed				

of the ingested glucose, 6 to 16 grams with an average retention of 10 grams per day.

Discussion. The average survival of animals subjected both to pancreatectomy and ligation of the lumbo-adrenal veins is 17.5 days for dogs and 19 days for cats, excluding the cat that lived 98 days. These averages are beyond those attained by untreated depancreatized animals. The average includes only those animals that survived 8 days or longer. Preparations that lived from 4 to 7 days were not studied intensively because animals subjected only to pancreatectomy usually succumb within that

period. However a distinct amelioration of symptoms is seen during this early period and usually sooner. The animals in which life was not prolonged may succumb to one of several causes. Grollman and Firor (3) originally noted that ligation of the lumbo-adrenal veins resulted in many instances in death from acute adrenal insufficiency. It is to be expected, therefore, that pancreatectomy superimposed on such a background would result in many fatalities. In some instances the amelioration of the diabetic symptoms was minimal and animals died with the characteristic picture of pancreas diabetes. However the prolonged survival period exhibited by many of the animals must be attributed to the effects of bilateral ligation of the lumbo-adrenal veins on the course of pancreas diabetes.

The variability of the blood sugar level has been noted in table 1. Values ranging from hypoglycemic to diabetic levels were observed. This variability may be explained by the nutritive condition of the animal and the degree of amelioration produced by the lumbo-adrenal vein ligations. It is interesting to note, therefore, that dog 15 which was well fed nevertheless had a normal post-absorptive blood sugar. The Houssay and the Long animals likewise present varying blood sugar levels.

The urinary studies of these animals are also similar to those previously reported for the Houssay and the Long preparations. Like the blood sugar, the glycosuria is variable. The average value of 0.83 gram per kgm. per day of urinary glucose for the fasted preparation is not much different from the values observed in the fed dogs. This close similarity between fasted and fed animals may be explained by the fact that there are a larger number of observations on fed preparations exhibiting marked amelioration. The greatest glycosuria 2.15 grams per kgm. per day is nevertheless definitely less than that observed for fed or even fasted depancreatized dogs. It may be added that the cats also exhibited a similar decrease of glycosuria and in many instances tests for urinary sugar were negative during fasting.

The diminished glucose excretion may be attributed in part to the decreased protein metabolism observed in both cats and dogs subjected to ligation of the lumbo-adrenal veins. The average nitrogen excretion for both fasted and fed dogs is about one-half that usually observed in pancreas diabetes. Similarly an increased oxidation of carbohydrate of these animals could undoubtedly account partly for the diminished protein metabolism. The decrease in urinary glucose, however, is more marked than that of nitrogen, yielding a D/N ratio lower than that of pancreas diabetes.

The data so far discussed suggest that this preparation is capable of utilizing some carbohydrate. The carbohydrate balance studies made on these animals support this conclusion. In the 20 observations made

on 6 animals, glucose was retained in significant amounts, from 6 to 16 grams per day. It is well known that depancreatized animals excrete practically all ingested carbohydrate. Thus ligation of adrenal veins permits the depancreatized animal to utilize, in some manner, ingested glucose. That these animals are capable of oxidizing carbohydrate is indicated by their total respiratory quotient which is elevated above that of fat oxidation to 0.79. The results presented in table 1, namely, the diminished glycosuria, nitrogen excretion, lowered D/N, as well as the retention of carbohydrate may therefore all be ascribed in part to the oxidation of carbohydrate by this preparation. Carbohydrate, however, is not oxidized in normal amounts. On the contrary, oxidation must proceed at low rate in comparison with normal, as evidenced by a glucose retention of only 10 grams (average) and a significant rise of the R.Q. after the ingestion of glucose in only 2 of 14 observations.

Heretofore it was believed that in the absence of insulin there could be no oxidation of carbohydrate. However more recently evidence has been accumulating against such a conception. (Himwich and Nahum, 7; Houssay, 1, and Chambers, Sweet and Chandler, 8.) We have here presented additional data disclosing that the ligation of the lumbo-adrenal veins also facilitates the oxidation of glucose in depancreatized animals.

The diminished ketonuria may also be ascribed partly to the increased oxidation of carbohydrate. This slight ketonuria is correlated with an average fat value raised only slightly above normal. Even in the animals exhibiting the higher levels of blood fat, the ketonuria was less than that of depancreatized animals without ligation of the lumbo-adrenal veins. In only one instance (not included in the average) has a hyperlipemia been observed comparable with that occurring in pancreas diabetes. The relatively low values for fat cannot be entirely attributed to an imperfect absorption of fat, as might be indicated by fatty stools since depancreatized animals with similar fatty stools nevertheless exhibit a high concentration of fat in the blood.

The question arises, how does ligation of the lumbo-adrenal veins ameliorate diabetes? There are several possible explanations. In the first place the venous congestion following the lumbo-adrenal vein ligation causes a diminished secretion of the cortical hormone, a secretion which probably enters the blood stream through unblocked venous anastomoses. This impairment may subsequently influence carbohydrate metabolism directly or indirectly. Much evidence has been presented which indicates that the adrenal cortex is directly concerned with carbohydrate metabolism. Britton (4) for many years has presented data to substantiate such a conception. The diminished blood sugar and liver glycogen occurring in adrenal insufficiency can be restored by injections of cortin and according to Britton this action of cortin constitutes its main function. It is

therefore surprising that Long's adrenalectomized depancreatized animals which received cortin in quantities sufficient to maintain life showed an amelioration of diabetes. It may be possible that his animals were in a state of chronic adrenal insufficiency. This possibility gains support from the latest work of Long (5) which demonstrates that larger doses of cortin injected into partially depancreatized rats produced an increase in the severity of the diabetes. Lukens and Dohan (6) have also demonstrated that huge daily injections of cortin, sometimes more than 40 cc., to the depancreatized adrenalectomized cat and dog result in an increased severity of the diabetic symptoms. Whether the injections of such large doses of cortin represent a pharmacological result is not at present known. However, it should be emphasized that the amounts of cortin injected are far in excess of that necessary to maintain the electrolyte pattern. From the evidence in the literature one might conclude the amelioration resulting from the ligation of the lumbo-adrenal veins is due to a chronic adrenal insufficiency. Nevertheless if we take as a criterion for deficient cortical function a lowered total base, our data disclose no consistent impairment. Despite a serum total base maintained within normal levels all our animals at some time exhibited an amelioration of the disturbances of carbohydrate metabolism. If the cortex does exert a direct diabetogenic effect on carbohydrate metabolism it must do so when present in amounts greatly in excess of those required to maintain the total base of the serum and to prevent the usual symptoms of adrenal insufficiency. Thus the influence of the adrenal cortex on carbohydrate metabolism does not appear to be directly related to the maintenance of the electrolyte pattern.

Ligation of the adrenal veins, according to Grollman and Firor (3), may produce a syndrome not only of chronic adrenal insufficiency but also of pituitary inadequacy; including cachexia, failure of growth and the development of the reproductive tract. These symptoms were relieved in their experiments by pituitary extracts and not by cortin injections. It is therefore possible that the improved carbohydrate metabolism is the result of a secondary effect in the anterior pituitary gland. Such an indirect influence is probable in animals surviving a long period. However some of our animals exhibited ameliorative effects within 2 to 4 days after the ligation. From the present results it is doubtful that the alleviation of the diabetes can be attributed either entirely to a diminution of cortin or to a secondary pituitary deficiency.

SUMMARY AND CONCLUSIONS

Thirteen dogs and 20 cats which were subjected simultaneously to bilateral ligation of the lumbo-adrenal veins and pancreatectomy revealed an ameliorated diabetes, as evidenced by prolongation of life, a lower blood sugar level, decreases in glycosuria, nitrogen excretion, ketonuria

and D/N. Blood fat was usually below the diabetic level. Carbohydrate balance studies revealed an average retention of 10 grams of glucose. This glucose is probably oxidized as indicated by a total R.Q. of 0.79. In many instances the total base content of the serum was within normal levels. Thus the change in carbohydrate metabolism does not appear to be secondary to that of inorganic metabolism. In conclusion, ligation of the lumbo-adrenal vein brings about an amelioration of pancreas diabetes in dogs and cats by an increased ability to oxidize carbohydrate. The mechanism for this amelioration is discussed.

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AN EXPERIMENT IN HUMAN DIETARY NIGHT-BLINDNESS

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Received for publication June 20, 1938

The earliest demonstrable symptom of vitamin A deficiency in man and other mammals is an impairment of visual capacities in dim light known as dietary night-blindness or hemeralopia.[2] This condition is associated with some failure in the dark adaptation processes which normally replace the retinal photopigments bleached in vision. The precise nature of the disturbance and of its dependence on vitamin A is still unknown, though all the components required for its investigation are now available. They include 1, the clarification of chemical and metabolic properties of vitamin A and its precursors among the plant carotenoids (Moore, 1933; Kuhn and Brockman, 1933; von Euler, Karrer and Zubrys, 1934; review by Zechmeister, 1937); 2, the demonstration that vitamin A participates with rhodopsin, the photosensitive pigment of the rods, in a retinal cycle (Wald, 1935–36); and 3, accurate information concerning the nature of human visual adaptation and its measurement (cf. Hecht, 1937; Hecht, Haig, and Chase, 1936–37; Wald and Clark, 1937–38). A thorough synthesis of this material should provide useful physiological information and a rational basis for the general clinical control of vitamin A deficiency.

The measurements presented below constitute an introductory attempt on the part of the authors to provide this information. They were performed by one of us (G. W.) at the Biological Laboratories of Harvard University, simultaneously with clinical and biochemical measurements by others to be reported elsewhere. H. J. initiated the deficiency experiment and supervised all dietary arrangements. J. A. served as subject throughout the research.

METHODS. Visual thresholds were measured with a specially designed adaptometer for which the instrument of Derby, Chandler and Sloan (1929) served as substrate. In this instrument the intensity of the test field may

[1] Aided in part by a grant from the Robert Dawson Evans Memorial, Boston.

[2] Clinical observations by Bloch (1921), Blegvad (1924), Birnbacher (1928), Aykroyd (1930); cf. review by Jeghers (1937a). Experimental studies on rats by Holm (1925), on cattle, sheep and pigs by Guilbert, Miller and Hughes (1937), and on a human subject by Jeghers (1937b).

be varied accurately over a range of one to several hundred thousand by means of a pair of circular neutral wedges rotating in opposite directions so as to compensate each other. Provision is made for inserting neutral and color filters into the path of the test light. In certain of the experiments in which deep red illuminations were required, a Wratten number *70* "monochromatic" red filter, which transmits only wavelengths above about 650 mμ, was used. In all other cases the full white radiation from a tungsten-filament incandescent lamp was employed.

The test field is circular and subtends a retinal angle of 2.1°. Above it is situated a small "star" of red light; when this is fixated, the image of the test field falls 11.7° above the subject's fovea. The fixation point is visible at all times. The test field is exposed by the subject for short flashes by means of a camera shutter set at 1/50 second. All observations are monocular; the field and fixation point are exposed alternately to the right and left eyes by displacement of a sliding diaphragm.

For *light adaptation* the subject faces a large opal-glass screen, mounted on top of the adaptometer. This is illuminated from behind with a 1000-Watt lamp, which may be withdrawn to various distances along a calibrated track to furnish the desired intensities.

A *standard procedure* was employed throughout the experiments. The subject was light adapted for precisely 4 minutes to a brightness of 4200 millilamberts. At the close of this period the adapting light was snapped off. The subject, now in complete darkness, fixated his eye and proceeded to flash the test field at intervals of about 1 second. The field was set initially at a sub-threshold intensity. Between clicks of the shutter the operator raised the intensity by regular steps of about 0.02 log unit (about 5 per cent) until the subject reported seeing the flash. The operator noted time and wedge-reading. Following the initial light adaptation, such thresholds were determined periodically in darkness, regularly alternating the two eyes.

This type of procedure, in which the subject can report his response to each intensity of test-light before it is raised, precludes "over-shooting" the threshold, and eliminates any unconscious tendency on the part of the operator to influence the results. The short exposures which we employ permit measurements of threshold to be spaced as closely as three per minute without appreciably affecting the course of dark adaptation.

The *subject* (J. A.) is a fourth-year medical student, age 26, normal weight 135 pounds. Some time previous to the present research he had been found to be markedly hemeralopic due to vitamin A-deficiency (Jeghers, 1937b, case 1). This condition was corrected by addition of milk, butter, fresh fruits, vegetables and vitamin A supplement to his diet. When the present experiments were begun the subject possessed perfectly normal visual adaptation and was otherwise in good health.

MEASUREMENTS. *Control period.* The research was begun with a control period lasting 48 days. During this interval the subject consumed a normal varied diet, supplemented with about 70,000 units daily of vitamin A.[3,4] About three times per week his dark adaptation was measured

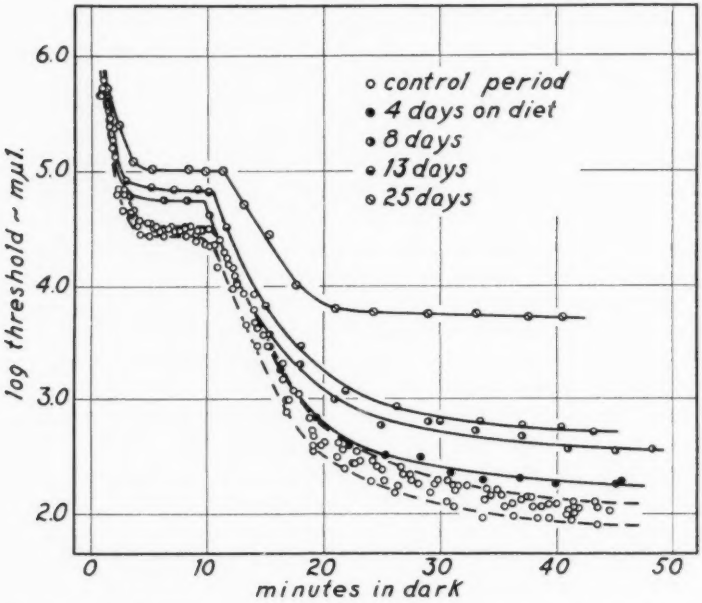

Fig. 1. Dark adaptation during a control period of optimal vitamin A nutrition (open circles; 6 experiments), and after various intervals on a diet deficient in vitamin A (closed and modified circles). Each point is a single determination of the threshold of the left eye to white light. The unit of threshold intensity is the milli-microlambert = 10^{-6} millilambert. Only abnormal portions of the data obtained on the fourth and eighth days of the deficient diet are shown.

with the standard procedure. The further course of the research can be evaluated only with close reference to these control data.

At the moment the adapting light is extinguished, the eye begins to

[3] Vitamin A and carotene quantities are expressed throughout this paper in the U. S. P. XI unit, equivalent to one International Unit (I.U.), or about 0.6γ of pure vitamin A or carotene.

[4] We wish to thank Parke-Davis & Co., Detroit, for supplying the halibut liver oil concentrates used as a source of vitamin A; the S. M. A. Corp., Cleveland, for carotene-in-oil and colloidal carotene preparations; and the Abbott Laboratories, North Chicago, for the brewer's yeast tablets, ascorbic acid, viosterol and calcium phosphate tablets used in these experiments.

increase in sensitivity, i.e., its threshold falls. When the logarithm of the threshold is plotted as a function of time in darkness, this change follows a characteristic course (fig. 1, open circles). There is an initial rapid fall to a plateau maintained from about the fifth to the tenth minute, then, following a sharp break, a second descent to a final plateau attained in about 40 minutes. It has been appreciated for some time that the initial segment is due to cones, the later portion to rods (cf. Hecht, 1937). The initial plateau therefore represents the threshold of the dark adapted cones, the final plateau that of the rods.

Cone and rod adaptations may be more completely separated by the use of deep red test lights, to which the rods are relatively insensitive (Hecht, 1937; Kohlrausch, 1931). In a number of experiments the standard light adaptation was followed with measurements of thresholds to "monochro-

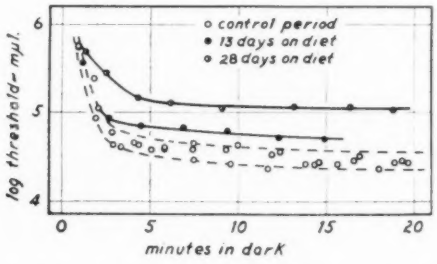

Fig. 2. Dark adaptation of the subject's left eye, measured with deep red test illuminations to which the rods are relatively insensitive. The data therefore describe the isolated adaptation of the cones. Otherwise as in figure 1. Data for the control period include 3 experiments.

matic" red light of wavelengths longer than 650 mμ. Under these conditions the dark adaptation of the cones may be followed in isolation for about 20 minutes. Data obtained in this way during the control period confirm those obtained with white light in showing cone dark adaptation to involve a fall in threshold of about 1.5 log units (about 32 times) and to be virtually complete within about 10 minutes (fig. 2, open circles).

The general expediency of plotting the visual thresholds in dark adaptation logarithmically has been appreciated for some time (Hecht, 1919-20; Kohlrausch, 1931, p. 1572). Two conditions give this usage special force in the present instance:

1. Each fall of one log unit represents a tenfold decrease in threshold. The normal range of dark adaptation in the present experiments is about 4 log units, or about 10,000 to 1. Following light adaptation the threshold falls to 1/10 of its initial value in about 2 minutes, to 1/32 (the cone plateau) in about 5 minutes, and to 1/100 in about 12.5 minutes. By this time, therefore, 99 per cent of the total absolute fall in threshold is already complete. This consideration has prompted the belief that

dark adaptation is "practically" over within about 10 minutes. Actually the further drop from 1 to 0.01 per cent of the initial threshold is as significant as the initial 99 per cent decrease, and is negotiated in about the same detail and with the same order of accuracy. This is a "practical" matter: when our subject's threshold failed to fall below 1 per cent, or even 0.1 per cent of its light adapted value, he was definitely night-blind, not only by clinical and laboratory test, but in his daily experience.

2. Dark adaptation data normally vary from day to day. Experimentally induced changes possess significance only with reference to this normal scatter. Figures 1 and 2 show this variation to assume very simple form on a logarithmic scale: it is approximately constant in width at all stages of cone and of rod adaptation. All data for the control period may be enclosed, therefore, between parallel lines. The day-to-day range of variation in each eye is about 0.1 log unit (about 26 per cent) for the cones, and about 0.2 log unit (about 60 per cent) for the rods (fig. 1).

Recently a large number of clinical observations have appeared concerning the behavior of normal and reputedly hemeralopic subjects when examined with the so-called "Biophotometer," using an arbitrary standard technique based upon visual adaptation (Jeans, Blanchard and Zentmire, 1937; Jeghers, 1937a, b). The relation of these to the present measurements can be stated only very roughly, since practically all phases of the procedures differ significantly. The conventional Biophotometer technique employs a 3 to 5 minute light adaptation to a brightness only about 1/21 of that used in the present experiments. This is followed with two measurements of threshold, at 20 seconds and at 10 minutes in darkness. Roughly these correspond with thresholds in the present procedure at about 8 and 18 minutes in darkness.

Rise of hemeralopia. At the close of the control period the subject abruptly eliminated vitamin A supplement and all milk products, eggs and colored vegetables from his diet. To ensure optimal supplies of all dietary factors other than vitamin A, the experimental regime was supplemented daily with 200 I. U. of vitamin B_1 and 150 Sherman units of vitamin B_2 taken in brewer's yeast, 50 mgm. of ascorbic acid, 2000 I. U. of vitamin D ("viosterol"), 8 grains ferrous sulphate, and 2 grams dicalcium phosphate. This basic diet, containing an estimated 50 to 200 units per day of vitamin A, had been found to induce hemeralopia within a short period (Jeghers, 1937b). It was maintained unaltered for 30 days. Its effects upon the course of dark adaptation are shown in figures 1 and 2; in figure 3 are plotted separately the thresholds of the completely dark adapted cones and rods (the cone and rod "plateaus").

At the close of 24 hours on this deficient diet, the first 30 minutes of dark adaptation of the right eye and the entire function in the left eye remained within the normal range. The threshold of the completely dark adapted right eye, however, had already risen significantly (fig. 3).

By the end of the fourth day, the final thresholds of both eyes had risen well above the normal range (figs. 1 and 3). The cone function and the

initial portions of rod dark adaptation still appeared normal. The threshold of the completely dark adapted eye, therefore, responds first to the vitamin A deficiency.

After six days on the deficient diet, a longer portion of rod dark adaptation had risen above the normal range, but the entire cone function still remained within it.

On the eighth day the cone plateau suddenly rose about 0.25 log unit (about 80 per cent) above the normal average. The rod plateau had by

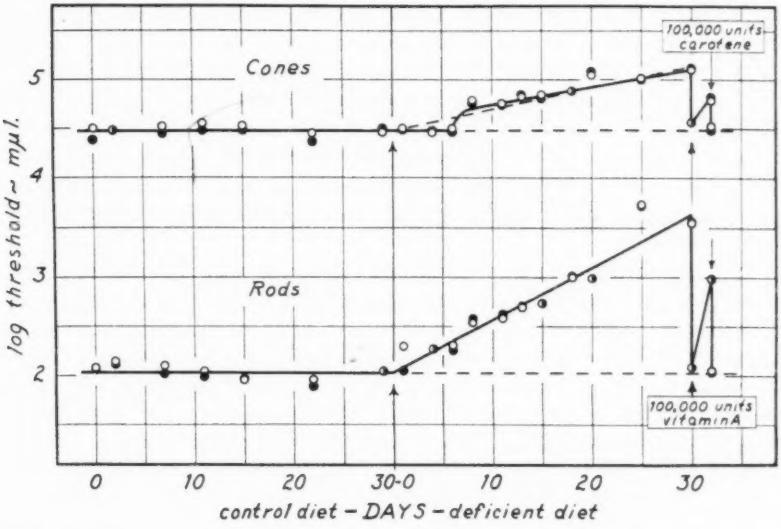

Fig. 3. Thresholds of completely dark adapted cones and rods (cone and rod "plateaus"), during 30 days of optimal vitamin A nutrition (left), and during 30 days on a vitamin A-deficient diet (right). Open and closed circles show thresholds of right and left eyes respectively. On the thirtieth day of the deficient diet vitamin A was administered; the course of cure is shown in figure 4*A*. On the thirty-second day the subject was again hemeralopic and was given carotene; the details of this experiment are shown in figure 5.

this time risen over 0.5 log unit (over 300 per cent) above the normal average. The initial portions of both cone and rod adaptation still remained within the normal range (figs. 1 and 3). It is true of the cones, as of the rods, therefore, that the completely dark adapted condition first shows the effect of the deficient diet. At this stage of the experiment thresholds measured between 4 and 10 minutes or after 15 minutes in darkness give clear evidence of hemeralopia, while those determined at other times are normal.

By the eleventh day the entire course of dark adaptation beyond the initial descending limb of the cone function had emerged from the normal range. Thereafter all thresholds continued to rise for the remainder of the deficiency period (figs. 1–3). Greater day-to-day variation marked the course of this development than was found during the normal period, probably due principally to fluctuations in the daily intake of vitamin A.

The most severe hemeralopia appeared in the rods on the twenty-fifth day, when their dark adapted threshold had risen about 1.7 log units or to about 50 times the normal average. The greatest change in the cones occurred on the thirtieth day, when their final threshold had risen 0.63 log unit, or to about 4.3 times the normal average.

It is clear that cones as well as rods become hemeralopic on the deficient diet. This is shown most unequivocally in the experiments with red test lights (fig. 2). The cones may respond later, and their thresholds rise more slowly than those of the rods. Nevertheless the cone response is of great theoretical and practical importance. It implies that all aspects of vision in dim light, including color vision, are impaired in vitamin A deficiency.

The rise of the rod plateau, plotted logarithmically, is described adequately with a straight line originating on the first day of the restricted diet (fig. 3). Most of the ascent of the cone plateau also is linear in this type of plot, following an abrupt rise on the eighth day. Indeed, a straight line, originating on the first day of the diet, may be drawn so that none of the cone data diverge from it further than is accounted for by the ordinary day-to-day variation. This is shown as a broken line through the cone data of figure 3. It is possible, therefore, that our apparent sudden onset of cone hemeralopia on the eighth day is a fortuitous effect of the scatter of the function, and that actually the hemeralopia of the cones originates on the first day of the deficient diet, as does that of the rods.

It has frequently been asserted clinically that the primary change in dietary hemeralopia is retardation in the velocity of dark adaptation with little or no change in its final level (cf. Dieter, 1931). This is not true in the present experiments. The data of figures 1 and 2 show that dark adaptation of both the cones and rods is completed as rapidly in the hemeralopic as in the normal eye. The primary abnormality is the rise of the cone and rod plateaus, and eventually of the thresholds at all levels of visual adaptation.

The threshold of the completely dark adapted eye not only first responds to the deficient diet; it systematically exhibits by far the greatest change. In the highly hemeralopic eye, as after the eleventh day in the present experiments, the log threshold diverges increasingly from normal as dark adaptation progresses. This observation is of great importance in the design of clinical procedures for the measurement of dietary hemeralopia.

Cure of hemeralopia. On the thirtieth day of the deficiency the subject's threshold at complete dark adaptation was found to be about 1.5 log units (about 32 times) above normal. He was given about 100,000 units

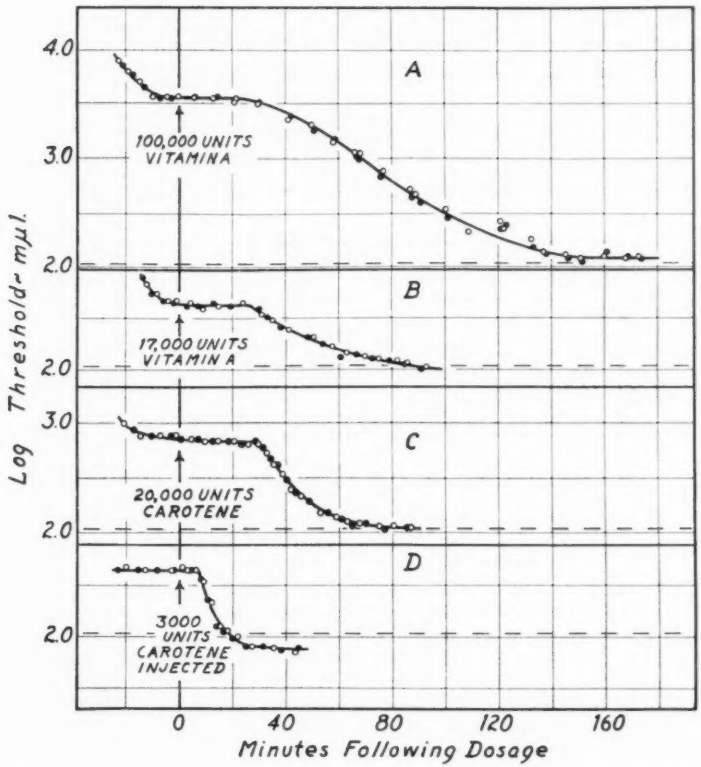

Fig. 4. Cure of hemeralopia with vitamin A and carotene. Thresholds of right and left eyes are shown as open and closed circles respectively. Measurements preceding the dosage show terminal portions of dark adaptation. In each section of the figure a broken line indicates the average threshold of the normal dark adapted eye. The threshold is markedly hemeralopic at the beginning of each experiment; after administration of vitamin A or carotene orally (*A-C*) or by intramuscular injection (*D*) the threshold descends to a final level within the normal day-to-day range.

of vitamin A in the form of 12 gelatine capsules of halibut liver oil, taken orally with water.

For about 24 minutes the dark adapted threshold remained unchanged. Then it began to fall regularly, and at 150 minutes following administra-

tion of the vitamin it had reached the normal level. The course of this change is shown in figure 4A. When the rod threshold had become normal, the subject was again run through the standard adaptation procedure. Both cone and rod segments of dark adaptation were found to be entirely normal (fig. 3).

Two days later, the deficient diet having been maintained unaltered, the subject was again decidedly hemeralopic (fig. 3 and 5). This second rise of hemeralopia was many times more rapid than its initial development. In these two days the cone plateau rose as high as in 8 to 12 days, and the rod plateau as high as in 18 days of the original deficiency. Presumably this great contrast in the effectiveness of vitamin A-deprivation

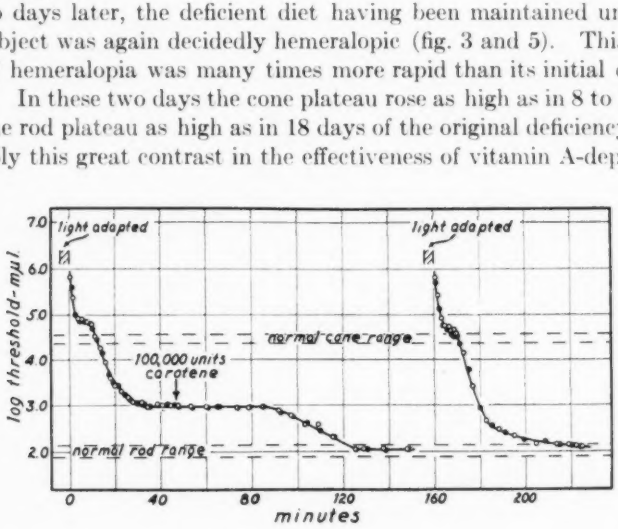

Fig. 5. Complete course of an experimental cure of night-blindness. Data of right and left eyes are shown with open and closed circles respectively. Following a standard 4-minute light adaptation, measurement of dark adaptation shows both cone and rod plateaus to lie well above their normal ranges (broken lines). When dark adaptation is complete, carotene is administered orally. After a "latent period" of 38 minutes, the rod threshold descends to normal. Repetition of the standard light and dark adaptation procedures shows both cone and rod plateaus to have entered the normal range.

is correlated with the presence of maximal vitamin reserves at the close of the control period, of little or no reserves at the end of the long initial deficiency. Since in both situations effects of the deficient diet were appreciable almost at once, it may be concluded that stored vitamin A in this instance retards the progress of hemeralopia, but does not defer its onset.

The entire course of the second "cure" experiment is shown in figure 5. The subject, when completely dark adapted, was given about 100,000 units of carotene in cotton-seed oil, taken orally in gelatine capsules with water. The hemeralopic threshold remained unchanged for 38 minutes. Then it descended rapidly and attained the normal rod level within 80

minutes following the carotene administration. Repetition of the standard adaptation procedure showed both cone and rod thresholds to have become normal.

During subsequent weeks the subject was repeatedly allowed to become hemeralopic on the deficient diet, and was brought back to normal temporarily by oral administration of carotene or vitamin A. The effects of these treatments are highly regular (fig. 4*A-C*, fig. 5; table 1). They begin with a "latent period" of 26 to 38 minutes during which the threshold remains constant. The duration of this interval is independent of the nature and concentration of the administered oil. It is probably occupied principally with solution of the gelatine capsules and delivery of the oils to the intestine.

TABLE 1

Numerical constants associated with the cure of dietary hemeralopia with various dosages and modes of administration of vitamin A and carotene

The "latent periods" in the fourth column are intervals between administration of the active principle and the first appreciable decrease in hemeralopic threshold. The numbers in the fifth column state the maximum fall in log threshold intensity per minute during the period of "cure."

ACTIVE PRINCIPLE	METHOD OF ADMINISTRATION	DOSAGE (U.S.P. XI UNITS)	"LATENT PERIOD"	MAXIMUM SPEED OF "CURE" (LOG THRESHOLD PER MINUTE)
Vitamin A	Oral	100,000	30	0.016
Vitamin A	Oral	17,000	26-30	0.016
Carotene	Oral	100,000	38	0.026
Carotene	Oral	20,000	30	0.033
Carotene (colloidal)	Intramuscular injection	3,000	7	0.068

The descent of the threshold to normal follows. Its velocity also is independent of the dosages we have employed. We find it, however, to be almost twice as rapid following oral carotene as after oral vitamin A administration. The maximum velocities of fall of log threshold in four "cure" experiments (fig. 4*A–C*; fig. 5), shown in table 1, illustrate clearly these characteristics.

Oral administration in gelatine capsules is obviously a very slow method for delivering the vitamin to the retina. Even the short periods for cure measured in the experiments described above must have been largely occupied with processes of digestion, absorption and transport. That this is true is demonstrated in an experiment in which 3000 units of colloidal aqueous carotene were injected intramuscularly into the gluteal region of the hemeralopic subject. The result is shown in figure 4*D*. Seven minutes following the injection the threshold broke sharply in descent. Within 20

minutes it had reached the normal rod level, and within about 35 minutes had become stable at as low a level as was ever attained by the normal eye during the control period.

This experiment shows that at least $\frac{3}{4}$ of the "latent period" following oral administration of the A factor and at least half of the period of "cure" are occupied with processes which deliver the vitamin to the tissues. Our observation that orally administered carotene acts more rapidly than vitamin A therefore is probably associated with greater efficiency in the absorption and transport of carotene, not necessarily at all with the efficiency of its utilization by the retina.

Discussion. Aykroyd's report in 1930 that marked dietary hemeralopia may develop within less than a month of vitamin A-deprivation and may be cured with one or two doses of cod-liver oil within 24 to 48 hours occasioned considerable interest. By 1932 the reported period for cure had been reduced to 12 to 24 hours.[5] With the introduction of sensitive procedures for estimating night-blindness, these times have since dwindled greatly. Jeghers (1937) observed evidences of hemeralopia on the sixth day of an experimental vitamin A deficiency, and some improvement of the hemeralopic threshold within 2 hours after oral administration of vitamin A. Edmund and Clemmesen (1936) have reported that following intramuscular injection of a vitamin A preparation the visual discrimination of three hemeralopic clinic patients became normal within from 7 to 10 minutes; the procedure employed, however, may be quite unreliable when applied as in this instance to untrained observers (cf. Groth-Petersen, 1938).

Our experiments show that an appreciable rise of visual threshold may appear within 24 hours on a vitamin A-deficient diet, in a normal subject previously optimally supplied with the vitamin. Pronounced hemeralopia may be improved within about 30 and cured within about 80 minutes after oral administration of vitamin A or carotene; intramuscular injection reduces these periods to about 7 minutes for improvement of threshold, and 20 minutes for complete cure. Even these very short intervals are probably limited principally by the method of administration of the vitamin.

It is necessary to distinguish clearly the acute "pure" vitamin A deficiency which we have studied from conditions more likely to be encountered outside the laboratory. Chronic vitamin A deficiency may involve widespread epithelial metaplasia (Wolbach and Howe, 1925) and nerve degeneration (Mellanby, 1934); and usually human malnutrition includes deficiencies in a wide variety of dietary essentials in addition to vitamin A. Hemeralopia in all these cases may be aggravated by anatom-

[5] Cited as a personal communication from Mori in the Medical Research Council Report on Vitamins (1932).

ical lesions which may respond to treatment comparatively slowly. To the extent, however, that a nutritional deficiency involves vitamin A, it includes a loss of visual pigments from the retina; and one initial effect of vitamin A therapy is the replacement of these pigments. These are the specific processes which we have studied. There is no present reason to believe that as components of more complicated situations their characteristics are altered. For these processes our observations, from their consistency with existing data, appear to furnish a reliable description.

Cones and rods. In the belief that hemeralopia is a specific disorder of the rods, Parinaud (1881) advanced it as a principal support of the "Duplicity" theory. Hess (1909) removed the acquired—as opposed to congenital—hemeralopias from this position, with the observation that in subjects hemeralopic from various causes, particularly in one case of chronic liver disease, the rod-free foveal area was pathological "in the same sense" as the rod apparatus. Hess' measurements were very crude by present standards. His conclusions, however, have recently been confirmed by precise measurements of dark adaptation in 13 persons afflicted with chronic liver diseases (Haig, Hecht and Patek, 1938). These determinations reveal an exact parallelism in the behavior of cone and rod thresholds in hemeralopia and in its response to vitamin A therapy.

The present experiments extend these observations to the field of "pure" vitamin A deficiency and its cure with carotene or vitamin A. In every instance complete parallelism in the behavior of cones and rods has been observed. The physiological significance of this situation is not yet certain. Numerous sharp differences in the physiological responses of cones and rods (Hecht, 1937), and the recent discovery of one specific photosensitive substance in cones (Wald, 1937) make it reasonably certain that cones contain photolabile pigments chemically distinct from rhodopsin. It is difficult to avoid the conclusion that vitamin A is the precursor not only of rhodopsin, but of some or all of the visual pigments in the human cones.

Vitamin A and rhodopsin. The dependence of adaptation in the rods upon vitamin A appears to be no more complicated than is implied in the equations of the retinal cycle: rhodopsin $\overset{\text{light}}{\rightleftharpoons}$ retinene-protein $\rightarrow$ vitamin A-protein $\rightarrow$ rhodopsin (Wald, 1935-36). Decrease in the concentration of available vitamin A results in corresponding decreases in retinene and rhodopsin, and consequent rise in the visual threshold. On readmission of vitamin A to the retina, rhodopsin apparently is at once synthesized to its normal concentration, and hemeralopia ceases. Dietary hemeralopia is essentially incomplete dark adaptation, its correction the completion of dark adaptation. It is probable that the limiting velocity with which hemeralopia might be cured, when vitamin A had been administered under

optimal conditions, should correspond with the concluding portions of a normal dark adaptation.

It is of some interest to estimate the absolute chemical significance of the changes which we have recorded. There is good reason to believe that the reciprocal of the dark threshold, as measured in the present experiments, is directly proportional to the concentration of rhodopsin.[6] If this is true, a rise in threshold of the dark adapted rods of 1 log unit (10 times) above the normal level implies a fall in rhodopsin concentration to $\frac{1}{10}$ its normal value. In his most extreme hemeralopia, therefore, our subject's dark adapted retina probably contained only about [redacted] of its normal complement of rhodopsin.

Carotene and vitamin A. Carotene introduced into the blood circulation is very rapidly and almost quantitatively absorbed by the liver (Drummond, Gilding and Macwalter, 1934). It is commonly supposed that carotene must be converted in the liver to vitamin A before being used by the tissues. This is generally assumed to be a disadvantage in the use of carotene as opposed to direct vitamin A administration. Our experiments show, however, that in a normal subject such disadvantage, if it exists at all, is over-compensated by the greater efficiency with which orally administered carotene is absorbed and transported. This observation probably possesses no practical importance whatever for the normal subject. However, in a number of cases of hemeralopia encountered clinically, oral administration of vitamin A has proved ineffective (cf. Edmund and Clemmesen, 1936). Apparently pathological conditions exist in which the absorption and transport of vitamin A become limiting factors in its utilization. It is possible that in such cases carotene possesses a significant therapeutic advantage.

The period between intramuscular injection of carotene and its effect on the visual threshold is so short as to suggest that the eye may use carotene directly. This possibility is subject to certain limitations. Though vitamin A has been found in all retinas so far examined which contain rhodopsin, none of them normally contains carotene (Wald, 1934–34; 1935–36). Also it is improbable chemically that carotene can substitute directly for vitamin A in the rhodopsin cycle. It must almost necessarily be assumed that if the eye can use carotene, some eye tissue in addition to the liver can convert it to vitamin A. This function might possibly be fulfilled by the pigmented epithelium, which is known to participate in rhodopsin synthesis, and often contains large quantities of the colored carotenoids (Wald, 1935–36).

[6] It is assumed that at the threshold a test flash of constant short duration, dt, decomposes a constant quantity of rhodopsin, dr. The rate of photolysis of rhodopsin is governed by the equation, $dr/dt = k\ I\ r$ in which k is a constant and I the intensity (Hecht, 1923–24). The assumption states that $dr = constant = k\ I\ r\ dt$, in which both k and dt are constants. Therefore $r = constant/I$.

Clinical procedure. Apparently the most appropriate visual measure of vitamin A deficiency is the threshold of the completely dark adapted eye. Only this threshold may conveniently be determined repeatedly without change, and the accuracy of the measurement consequently increased enormously by repetition and averaging. This threshold responds first and most to vitamin A deficiency, and appears to be the most sensitive available measure of hemeralopia.

We suggest, therefore, that in future clinical procedures for estimating dietary night-blindness, preliminary light adaptation be omitted. Subjects may be dark adapted in a darkened room or by some other device for withholding light from the eyes. Following 30 to 45 minutes in darkness, the visual threshold may be determined repeatedly. The scatter of the measurements provides a clear indication of the reliability of the subject's reports. Their average, a single number, should constitute the most exact index now available of the hemeralopic state, and therewith of the vitamin A nutritional level.

SUMMARY

1. The dark adaptation of a human subject was measured at regular intervals during a long control period; and during a subsequent period on a diet containing only 50 to 200 U. S. P. units daily of vitamin A, but otherwise complete.

2. A first effect of the deficient diet was noted within 24 hours. Within 25 days the threshold of the dark adapted rods had risen about 50 times, that of the dark adapted cones about 4 times. The threshold of the dark adapted rods responds first and most strongly to the vitamin A deficiency.

3. Following temporary cure of the initial night-blindness with a single dose of vitamin A, hemeralopia re-appeared with greatly increased rapidity, presumably due to depletion of vitamin A reserves during the initial deficiency.

4. The development of hemeralopia was repeatedly checked temporarily by oral administration of vitamin A or carotene. Ingestion of either of these is followed by a "latent period" of about 30 minutes during which the hemeralopic threshold remains unchanged. Then the threshold descends to normal, about twice as rapidly after carotene as after vitamin A administration. This difference in effectiveness appears to be due to greater efficiency in the absorption and transport of carotene.

5. Following intramuscular injection of colloidal carotene the hemeralopic threshold improved within 7 minutes and fell to normal within 20 minutes.

6. In all these changes the behavior of cones parallels that of rods. Vitamin A appears to be the precursor of cone visual pigments as well as of the rhodopsin of the rods.

7. It is suggested that clinical measurements of hemeralopia omit preliminary light adaptation of the subject and accept the threshold of the completely dark adapted eye as the most satisfactory hemeralopic index.

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THE DIFFUSION OF GLUCOSE AND SUCROSE FROM THE CEREBROSPINAL FLUID

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Received for publication June 21, 1938

Recent investigations (Bullock, Gregersen and Kinney, 1934, 1935; Masserman, 1934a) have shown that hypertonic solutions of glucose and sucrose given intravenously have markedly different final effects upon cerebrospinal fluid pressure. Both cause an initial fall in intracranial pressure. In the case of glucose this is followed by a rise above the pre-injection level, whereas in the case of sucrose, the pressure rises more gradually and does not exceed the original level. The explanation generally given for the secondary rise is that the substances which produce it diffuse readily into the cerebrospinal fluid and brain, which then remain hypertonic and take up water when the concentration in the blood stream falls (Fay, 1924; Masserman, 1934a). Sucrose presumably fails to evoke the so-called "secondary rise" because it is unable to penetrate the blood-spinal fluid barrier. Analyses of fluid collected from the cisterna magna of dogs immediately before and 1–3 hours after the intravenous injection of sucrose confirmed this view (Gregersen and Wright, 1935). Very little if any sucrose (measured as hydrolyzable reducing substance) could be found in the spinal fluid. On the other hand, when comparable quantities of glucose were injected, there was a large increase in concentration of this substance in the cisternal fluid.

The purpose of the experiments reported here was to find out if this difference in the permeability of the blood-spinal barrier to glucose and sucrose could be demonstrated by measuring the rate of their passage in the reverse direction; namely, from cerebrospinal fluid to blood. Measured amounts of hypertonic solutions were injected into the cisternal space of dogs and the fraction of the sugars remaining within the subarachnoid space at various times thereafter estimated from *a*, the changes in spinal fluid pressure following the injection, and from *b*, chemical analyses of the fluid.

PROCEDURE AND RESULTS. A. *Hydrostatic pressure measurements.* Under aseptic conditions, a large hypodermic needle, connected by means

of a three-way stopcock to an open manometer (1 mm. diameter[1]) was thrust into the cisternal space of a dog anesthetized with nembutal. After recording the normal pressure for a period of one hour, 2 cc. of fluid were withdrawn and an equal volume of a sterile solution of the desired substance immediately injected in its place. The rise and subsequent fall in pressure were then observed during the following 2 to 3 hours. If the control period showed a large rise in pressure indicating a possible septic meningitis or irritative meningismus the experiment was discontinued.

According to the experiments of Flexner and Winter (1932), the cerebrospinal fluid is apparently secreted quite slowly under normal conditions of pressure. Reduction of the intraventricular pressure however greatly facilitates fluid formation. The question therefore arose as to whether or not the abrupt increase in pressure observed after the replacement of a measured amount of cisternal fluid with an equal volume of hypertonic sugar solution might not have been caused in part by a more rapid forma-

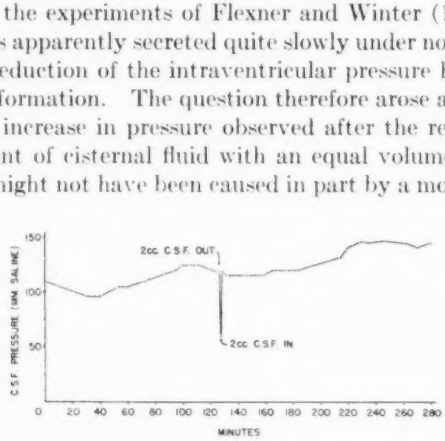

Fig. 1. Control experiment showing the effect on cerebrospinal fluid pressure of withdrawal and reinjection of 2 cc. of fluid.

tion of cerebrospinal fluid during the two or three minutes of low pressure existing while the replacement was being carried out. Masserman (1934b) reports that repeated withdrawal and reinjection of spinal fluid in human subjects leads to a progressive rise in spinal fluid pressure. Under the conditions of the experiments described here, however, there was no significant rise in pressure when 2 cc. of fluid were withdrawn and reinjected after 2 or 3 minutes (fig. 1).

It may be seen from the typical experiments presented in figure 2 that 2 cc. of a hypertonic sucrose solution (50 per cent) produces a much longer and more pronounced increase in cerebrospinal fluid pressure than does an approximately iso-molar amount of glucose (2 cc. of a 25 per cent solution), indicating that sucrose remains within the spinal fluid longer than does glucose. Nevertheless, direct comparison of the hydrostatic changes

[1] With a manometer of this bore, dislocation of fluid with changes in pressure does not introduce significant errors (Weed, Flexner and Clark, 1932). A rise of 100 mm. displaces only 0.08 cc. of fluid.

produced by the cisternal injection of these sugars is difficult for two reasons: 1, the initial pressure varies in different animals, and 2, the concentration of the sugar in the space cannot be established at the same level in every experiment since the volume of cerebrospinal fluid in the intact animal is not measurable. In order to meet these difficulties and to obtain clearer evidence of the relative rates at which the sugars escape from the spinal fluid, the pressure curves have been reduced to the same scale. This simplification is possible if the maximal increase of pressure caused by the injection be defined in each case as 100, and the height of the pressure above the control level at any time thereafter be plotted as per cent of the maximal rise.

The results of treating the data in this manner are presented in figure 3. These curves presumably show that in comparison with the amount of the injected sugar present when the pressure is maximal, the percentages of glucose and sucrose remaining 60 minutes later are respectively 64 and 38. The ratio of the per cent sucrose to glucose left is 1.69.

It is possible that glucose disappears more rapidly than sucrose not only because it diffuses more readily out of the spinal fluid, but because it is removed and metabolized by the brain tissue. For this reason, galactose was also tested. This sugar is not metabolized by the brain in significant amounts (Roe and Cowgill, 1935). Figure 3 shows that the galactose leaves the cerebrospinal fluid even more rapidly than glucose. Destruction of the glucose in the cerebrospinal fluid does not therefore explain its rapid disappearance in comparison with that of sucrose.

B. *Chemical determinations.* In order to measure the diffusibility of glucose and sucrose under exactly similar conditions, the sugars were injected simultaneously in a solution containing approximately equivalent concentrations of each. The procedure was as follows: A sample of spinal fluid was removed to determine the amount of reducing substance already present. A solution containing about 12.5 per cent sucrose and 6.5 per cent dextrose was then injected into the cisternal space. Five minutes later a second sample was withdrawn and about an hour later a third. Duplicate determinations of the dextrose and sucrose concentrations in each sample were made with a modification of Folin's micro-method (Gregersen and Wright, 1935).

It should perhaps be pointed out that these experiments demonstrate only the relative rates of disappearance of dextrose and sucrose from the fluid immediately surrounding the needle in the cisternal space, and that a part of the decrease in concentration which occurred may have been caused by diffusion within the cerebrospinal fluid system itself. However, Dixon and Halliburton (1916) demonstrated that if sodium salicylate or other crystalline substances are injected intracisternally, they appear in the blood of the confluens sinuum within 5 minutes. Therefore, it is

TABLE 1

Results, as determined by chemical analysis, showing the relative amounts of sucrose and glucose remaining in the cisternal space for a period of 60 to 75 minutes

EXPERIMENT	TIME BETWEEN WITHDRAWAL OF 2ND AND 3RD SAMPLES	SUGAR	1ST SAMPLE (CONTROL)	2ND SAMPLE (EXOGENOUS SUGAR)	3RD SAMPLE (EXOGENOUS SUGAR)	PER CENT SUGAR REMAINING $\left(\frac{\text{3RD SAMPLE}}{\text{2ND SAMPLE}} \times 100\right)$	RATIO PER CENT SUCROSE PER CENT GLUCOSE
	min.		*mgm./ per cent*	*mgm./ per cent*	*mgm. per cent*		
1	60	Sucrose		678	512	76	1.90
		Glucose	67	217	87	40	
2	65	Sucrose		435	362	83	1.78
		Glucose	70	196	92	47	
3	75	Sucrose		1,454	719	49	1.88
		Glucose	76	586	154	26	
4	75	Sucrose		714	497	69	1.72
		Glucose	67	273	108	40	
Average							1.82

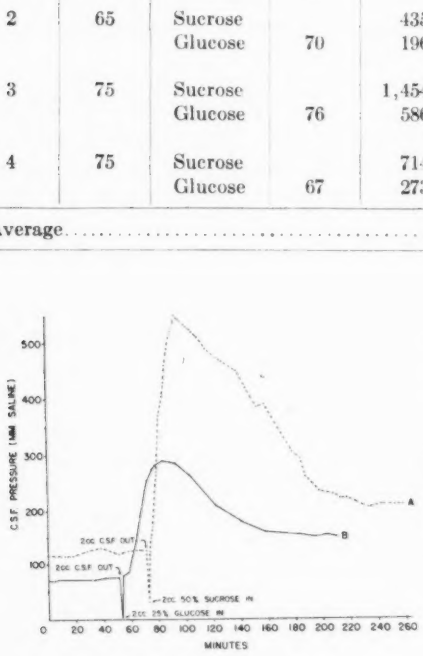

Fig. 2

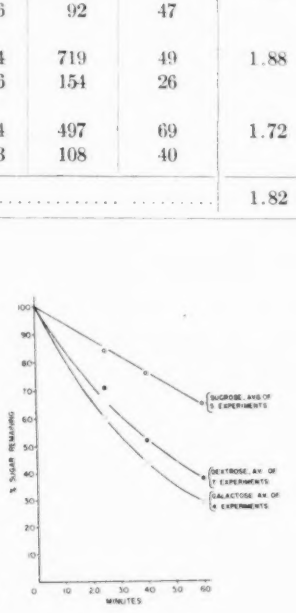

Fig. 3

Fig. 2. Changes in cerebrospinal fluid pressure following the cisternal injection of *A*, 2 cc. of 50 per cent sucrose (dog 7.5 kgm.), and *B*, 2 cc. of 25 per cent glucose (dog 10.0 kgm.).

Fig. 3. Per cent of injected sugar remaining within the subarachnoid space estimated from the changes in cerebrospinal fluid pressure (see text).

probable that sufficient mechanical mixing occurs in the cerebrospinal fluid to disperse both sugars equally, although in a solution at rest more sucrose than glucose would remain near the locus of injection because of the difference in the diffusion rates of the two sugars.

The results obtained have been tabulated in table 1. It may be seen that although the relative amount of sugar remaining is by no means the same in all experiments, the ratio of per cent sucrose to glucose remaining after 60 to 75 minutes is quite consistent. Furthermore, the average ratio, 1.82, corresponds fairly closely to the ratio 1.69, estimated from the hydrostatic pressure changes described earlier. Both series of experiments thus lead one to the conclusion that glucose diffuses out of the spinal fluid more rapidly than does sucrose.

The results reported by Gregersen and Wright (1935) indicate that the blood-spinal fluid barrier is practically impermeable to sucrose. In the experiments described here we have seen that when sucrose is injected intracisternally, a large fraction of it disappears, presumably into the blood stream, within a comparatively short time (see fig. 3 and table 1); several tests revealed only traces of sucrose in the fluid 24 hours after the injection. This difference in the results of the two series of experiments might on superficial examination be interpreted to mean that the permeability of the blood-spinal fluid barrier to sucrose is greater in one direction than in the other. It seems more likely, however, that the discrepancy may be attributable to differences in the sucrose concentration-gradients and the hydrostatic pressure relationships between blood and spinal fluid.

CONCLUSIONS

1. The monosaccharides, glucose and galactose, leave the subarachnoid space more rapidly than does the disaccharide, sucrose.

2. Since it is known that galactose is not metabolized by the brain (Roe and Cowgill, 1935) the similarity in the rates of disappearance of galactose and glucose from the subarachnoid space suggests that glucose in the spinal fluid is not used by the brain in appreciable amounts.

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THE INFLUENCE OF CALCIUM AND POTASSIUM SALTS ON THE UTERINE CONTRACTIONS IN NORMAL AND ADRENALECTOMIZED RABBITS[1]

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Received for publication May 11, 1938

Data concerning the sensitivity of the uterus to calcium and potassium ions has been derived, for the most part, from two types of experiments: studies on excised uterine strips, and in acute experiments on the uterus *in vitro*.

Bacq and Rosenbleuth (3) reported a series of experiments of the acute type on the effect of intravenous injections of calcium chloride and potassium chloride on the non-pregnant uterus of the cat. They concluded that the uterine response to these ions was due to their stimulative effect on the adrenal medulla. When both adrenal glands were left intact, calcium and potassium salts had an inhibiting action on the contracting uterus. These ions, however, caused an increase in the contractions when the adrenal blood supply was cut off. The secretion of adrenalin, which was said to cause the inhibition of the uterus, was shown to be due to both peripheral and central stimulation of the gland. The very nature of such experiments, however, makes it impossible to duplicate normal physiological conditions with any great degree of accuracy.

The excellent technique developed by Reynolds (11) and Reynolds and Friedman (12) makes it possible to record the contractions of the intact uterus *in situ*. Experiments may be continued over a period of days on the same animal, and there is no necessity for anesthesia.

Weinstein and Friedman, as reported by Friedman and Friedman (7), found that as much as 8 cc. of calcium gluconate (10 per cent) injected at one time, elicited no response in the rhythmically contracting uterus of the rabbit, as recorded by an intra-uterine balloon. Reynolds (13), on the other hand, obtained sustained contractions of the rabbit uterus in response to injections of calcium chloride or calcium gluconate (10 per cent) in amounts varying from 0.5 cc. to 12 cc., provided the uterus was con-

[1] The material presented here represents part of the dissertation of the senior author, Sylvia B. Rouse, presented to Bryn Mawr College as partial fulfillment of the degree of Doctor of Philosophy.

tracting rhythmically prior to the injection. Both intravenous and subcutaneous injections were effective. So far as is known, the effect of the potassium ions has not been recorded by this method.

It seemed advisable to investigate the action of the calcium and potassium ions on the uterus under normal physiological conditions, viz., by means of the uterine fistula, and to check the rôle of the adrenal gland in the uterine response to these ions. The effects of intravenous injections of calcium and potassium chloride on the rhythmically contracting uterus in normal and adrenalectomized rabbits have therefore been studied.

MATERIALS AND METHODS. The rabbits used in these experiments were does which had previously had a litter. The records of the contractions of the uterus were obtained by means of a balloon inserted in a uterine fistula, according to the method developed and described by Reynolds (11). They were all bilaterally oöphorectomized, and at the time of the experiments were receiving daily injections of Theelin (Parke, Davis).[2] Records of uterine contractions were made on three groups of animals: normal, unilaterally adrenalectomized (physiologically normal), and bilaterally adrenalectomized rabbits.

Operative technique. Adrenalectomy was performed in two stages through longitudinal incisions starting at a point just between the ends of the tenth and eleventh ribs. The gland was carefully separated from the vena cava by blunt dissection, and the stump attaching the gland to the vena cava ligated by passing a ligature under a curved hemostat placed between the gland and the blood vessel. The gland was then cut away above the hemostat and examined to assure complete removal.

Anesthesia for all of the operations was produced by intravenous injections of Nembutal (Abbot Laboratories) in doses of 1 cc. per 5 lbs. of body weight. This was followed by ether when necessary. Aseptic technique was employed throughout.

It was found that careful post-operative treatment was essential, in order that the animals would not undergo severe surgical shock resulting in death. This was especially necessary after the removal of the second gland. Immediately upon the completion of each operation, the animals were given 20 to 30 cc. of warm, sterile Ringer's solution intravenously. They were then put into warm covered cages, and left undisturbed for twenty-four hours.

The success of the operations was determined by the appearance of typical symptoms of adrenal insufficiency followed by death. Death within twenty-four hours after an operation was attributed to surgical shock. Each animal was autopsied and examined for infections, adhesions, lung inflammation, etc. The results reported in this paper are taken only from records of those animals which developed symptoms of insufficiency followed by death, and which were shown by autopsy to be otherwise in good health.

The uterine fistula was put in according to the operating procedure of Reynolds (11).

The apparatus used to record the uterine contractions was that described by Reynolds (11).

[2] We are indebted to Dr. Oliver Kamm, Research Director of the Parke, Davis Co., for the supply of Theelin used in these experiments.

It has been shown by Reynolds (13) that the rabbit uterus responded to the injection of calcium salts only when the uterus was contracting rhythmically. Because this motility of the uterus is determined by the sexual state of the animal (Reynolds, 14), the rabbits were bilaterally oöphorectomized at the time of the fistula operation, and each animal was injected with 25 to 50 R.U. of Theelin twenty-four hours before each experiment. Such an animal is referred to as a "standard" animal, and, in respect to the motility of the uterus, is in a conditon equivalent to oestrus.

Solutions. Five per cent solutions of calcium and potassium chloride were used in this work. In the preliminary experiments it was found that the uterine response to injections varied not only with the amount injected, but also with the rate of injection. This has also been noted by Reynolds (13). It was found that 2 cc. of 5 per cent calcium or potassium chloride injected intravenously over a period of one minute caused a marked change in the character of the uterine contractions. This change could be reproduced by similar injections not only in the same animal on different days, but also in other animals. Since the purpose of the experiments was to compare the difference in the response of the uterus in normal and adrenalectomized rabbits, this standard dose and injection time was employed in most of the experiments.

Experimental procedure. The normal records reported here were obtained from two sources:

1. Animals in which both adrenal glands were intact.

2. Animals in which one adrenal gland had been removed. In this case the normal records were taken prior to the removal of the second gland. This made it possible to obtain records on the same animal before and after the removal of the second gland.

The adrenalectomized rabbits were, of necessity, subjected to a rigorous operative routine. The schedule for each rabbit was subject to a variation of from one to three days in the length of time elapsing between operations. The usual schedule, however, was as follows: The right adrenal gland was removed. One week later the animal was oöphorectomized and the fistula put in. Nine days later the left adrenal was removed.

Results. The results given here are based on sixty-five separate observations over a period of a year. Of these observations, twenty-two were made on seven normal rabbits, and thirty-three were made on seven bilaterally adrenalectomized rabbits.

Examples of the records obtained are given in the plate. *A* and *B* are taken from the results on normal rabbits, while *C* and *D* show the uterine response after bilateral adrenalectomy.

During the experiments, injections of Ringer's solution were made in order to show that the uterine response to the salts was not due to a direct response to the injection of fluid. The injection of 2 cc. Ringer's solution

at the same rate as the Ca and K salts, produced no change in the uterine contractions.

The results obtained indicate that the response of the rabbit uterus to calcium and potassium ions is not affected by the loss of the adrenal glands, and therefore not affected via the adrenal glands through epinephrine. In none of the observations did the uterine response of the normal rabbits differ from that of the adrenalectomized rabbits. No inhibition or relaxation of the uterus was noted. The injections of 2 cc. calcium or potassium chloride at the rate of 2 cc. min. produced a sustained contraction of the uterus, whether the animal had one or both adrenals intact, or had been bilaterally adrenalectomized.

Discussion. The records obtained in this series of experiments show that in the rhythmically contracting rabbit uterus *in situ*, calcium and

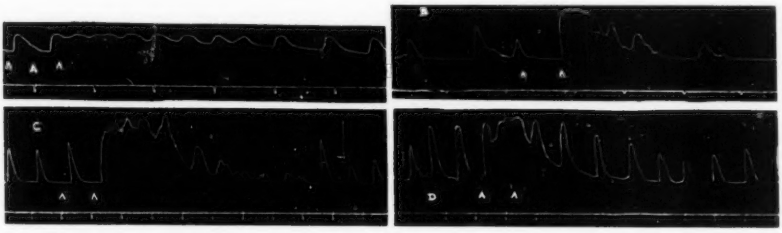

Fig. 1. The bottom line gives time in minutes; the arrow marks show periods of injection.

A. Normal rabbit, bilaterally oophorectomized; 2 cc. 5 per cent $CaCl_2$.

B. Normal rabbit, bilaterally oöphorectomized, unilaterally adrenalectomized; 2 cc. 5 per cent KCl.

C. Rabbit bilaterally adrenalectomized, bilaterally oophorectomized; 2 cc. 5 per cent $CaCl_2$.

D. Same as *C*; 2 cc. 5 per cent KCl.

potassium ions produce a sustained contraction of the uterus. Bacq and Rosenblueth (3) obtained similar results in the denervated cat uterus provided the adrenal glands were ligated; when the adrenal glands were left intact, however, both calcium and potassium ions inhibited the uterine contractions.

Bacq and Rosenblueth attribute this inhibition to the action of adrenalin. After removing one or both sympathetic chains above the kidney they reported that calcium and potassium ions cause the secretion of adrenalin, and that the stimulation of the chromaffin cells was caused both by a peripheral stimulation and by a central stimulation via the splanchnics.

The experiments here reported do not show any inhibition of the contracting uterus after injection of calcium or potassium ions, nor do they indicate that the adrenal gland is involved in the response of the uterus to

these ions. In this comparison of experimental results, two possible causes for the disagreement should be pointed out. In these experiments on the rabbit a "standard" animal has been used, while in Bacq and Rosenblueth's experiments the sexual state of the animal is not reported, other than that the uterus is non-pregnant. The importance of the condition of the rabbit uterus has been cited previously (Reynolds, 13, 14). This has been discussed further by Sauer et al. (15). Heller and Holtz (10) had shown that the condition of the isolated uterus of the guinea pig affects its response to adrenalin. They found that during oestrus adrenalin caused the uterus to contract, whereas during dioestrus a relaxation was produced. The species difference between the cat and rabbit uterus in response to adrenalin should also be mentioned. Adrenalin causes inhibition of the non-pregnant cat uterus and a stimulation of the non-pregnant rabbit uterus (in a state equivalent to oestrus).

In these experiments on the rabbit uterus, calcium and potassium salts elicited a sustained contraction of the uterus in both the normal and adrenalectomized animals. It is difficult to make a quantitative comparison of the uterine response in this type of experiment. Nevertheless, the experiments indicate that in the adrenalectomized rabbits, the uterus shows no lessened response to the calcium and potassium ions, as would be expected if the secretion of adrenalin were a factor in the uterine response to these ions. It therefore seems clear that in the results reported here, the secretion of adrenalin is not involved.

In the light of the failure to demonstrate any antagonistic action between calcium and potassium upon uterine contractions, it seems possible to take these results as further evidence against the theory that an alteration in the Ca/K ratio plays a rôle in sensitizing the uterus to pituitrin at the time of parturition (Heller and Holtz, 10).

A possible connection between adrenal cortical function and the maintenance of the normal female sex cycle has been suggested and denied by many workers (Anderson and Kennedy, 1, 2; Wyman, 16, 17; Corey and Britton, 5, 6; Cleghorn, 4; Gaunt and Parkins, 8; Gaunt and Tobin, 9). The uteri of all these adrenalectomized and oöphorectomized rabbits receiving Theelin, showed normal, rhythmic contractions up until death. It therefore seems unlikely that the presence or absence of the adrenal cortex has any effect upon the normal response of the rabbit uterus to the oestrogenic hormone.

CONCLUSIONS

1. The effect of intravenous injections of KCl and $CaCl_2$ on the uterine motility in normal and adrenalectomized rabbits has been studied.

2. KCl and $CaCl_2$ produced sustained contractions of the uterus *in situ* in both normal and adrenalectomized rabbits.

3. The effect of changes in Ca/K ratio at parturition has been discussed.

4. Theelin maintains the normal uterine motility typical of oestrus in the absence of adrenal cortical tissue.

Acknowledgments. We are indebted to Dr. S. R. M. Reynolds of the Long Island College of Medicine, who generously helped us in developing the uterine fistula technique, and to Mr. John Bookman and Miss Eleanor Yeakel for their assistance in some of the operations.

The portion of this work done at the Biological Laboratory, Cold Spring Harbor, N. Y. was aided by a scholarship granted to one of us (S. B. R.) by that institution.

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EXPERIMENTAL EVIDENCE SUPPORTING THE CONCEPTION OF "ADAPTATION ENERGY"

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Received for publication May 23, 1938

It has been shown that when an organism is exposed to a stimulus to the quality or intensity of which it is not adapted, it responds with a reaction which has been termed the "general adaptation syndrome" (Selye, 1936a, 1937a, 1938a). The symptoms of this syndrome have been described elsewhere (Harlow and Selye, 1937; Howlett and Browne, 1937; Karády et al., 1938; Schacher et al., 1937; Selye, 1936b, 1937b, 1937c; Selye et al., 1936). They are largely independent of the specific nature of the agent to which adaptation occurs, so that the reaction has been regarded as the somatic expression of damage as such. The general adaptation syndrome develops in three distinct stages which have been termed: 1, the stag of the alarm reaction; 2, the stage of resistance, and 3, the stage of exhaustion. The symptoms of the alarm reaction, among which thymus atrophy and adrenal hyperplasia are particularly conspicuous, disappear during the stage of resistance in spite of continued treatment with a uniform damaging stimulus (a drug, exposure to cold, excessive muscular exercise, etc.) but these same symptoms reappear during the so-called stage of exhaustion and finally death ensues. A similar three stage reaction was recently observed by Giragossintz and Sundstroem (1937) in rats kept under low oxygen tension. In these experiments, the loss of acquired adaptation during the stage of exhaustion is difficult to explain but as a working hypothesis, it was assumed that every organism possesses a certain limited amount of "adaptation energy" and once this is consumed, the performance of adaptive processes is no longer possible (Selye, 1938c).

Previous observations (Karády et al., 1938; Selye, 1938a, 1938b) showed that during the alarm reaction, the resistance of the organism is increased, not only to the stimulus with which the alarm reaction had been elicited but also to agents of a different nature. However during the second stage of the adaptation syndrome, this non-specific resistance vanishes rapidly at a time when the specific resistance to the agent with which the animal had been pretreated, is still very high. These findings

are in accord with the conception of adaptation energy. Yet we must realize that this conception is quite contrary to common belief, since it is generally agreed that all vital processes are performed merely at the expense of the caloric energy of the ingested food. We felt, therefore, that further experimental evidence is required before one could regard it as proven that some principle not replaceable by food intake is expended during the process of adaptation. The two most important facts capable of proving that such a principle is utilized during adaptation and that the organism possesses only a limited amount of this "adaptation energy" are that acquired adaptation vanishes after a certain time and that while an organism builds up resistance against a certain agent it loses much of its ability to resist agents of a different nature. The object of this communication is to report on new experiments proving these points.

As we said above, a decrease in the weight of the thymus is a very sensitive index of damage caused by stimuli capable of eliciting an alarm reaction. We thought, therefore, that the weight of this organ would give us an objective indicator of the resistance to such stimuli. But the thymus involution produced by a stimulus during the alarm reaction may persist for quite some time even after the animal has entered into the stage of resistance, so that we had to devise a method by which one could establish resistance without first going through a marked initial stage of alarm. We found this to be possible if one uses gradually increasing doses of the alarming stimulus. Under these conditions, one may finally administer the "full alarming dose" (which has been defined as the dose just sufficient to produce a marked alarm reaction within 48 hours after the beginning of the experiment (Selye, 1937a)) without producing thymus involution. Animals thus treated are therefore led into the stage of resistance while they still possess a well developed thymus gland which may act as a sensitive indicator of the damage caused by various agents. Since short pretreatment with rapidly ascending doses would be insufficient to prevent an alarm reaction while prolonged pretreatment with very slowly ascending dosages may lead to the stage of exhaustion before the full alarming dose is reached, we performed a series of experiments, the purpose of which was to establish the optimum length of pretreatment which would prevent thymus involution following the administration of the full alarming dose of a stimulus. Table 1 gives an example of some experiments of this sort in which adrenaline was used as the damaging agent. The full "alarming dose" of this drug for a rat 3 months of age was found to be 0.2 cc. of a 1 per cent solution given subcutaneously twice daily during two subsequent days. In all these experiments we began treatment with $\frac{1}{4}$ of this dose and gradually raised it to the above mentioned amount which was then administered during the last two days. The italic numbers represent the average thymus weight of a group of 6 female rats, 3 months of age, the

numbers in parentheses being the extreme variations observed. It will be remembered that all of these animals received during the last two days the same amount of adrenaline as the two day group which was simply treated with the full alarming dose without pretreatment. In the three day group (that is, one day pretreatment), or even more in the four day group (two days' pretreatment), the pretreatment actually increased the thymus involution elicited by the alarming dose administered during the last two days of the experiment. In these short run experiments, apparently the effect of the pretreatment was simply superimposed on that of the alarming dose, since there was not sufficient time for adaptation to develop. In the very chronic experiments, on the other hand, the effect of adaptation was lost because of exhaustion. The data show that in the case of adrenaline starting with one quarter of the alarming dose, the least damaging way of establishing resistance is to raise the daily amount to the full alarming dose within 5 to 12 days. If the daily dose is in-

TABLE 1

Importance of the length of treatment on establishing resistance of rats against adrenaline

PERIOD OF TREATMENT	THYMUS WEIGHT
days	*mgm.*
2	*127*, (107, 205)
3	*100*, (73, 126)
4	*80*, (54, 150)
5	*180*, (112, 227)
8	*230*, (180, 254)
12	*174*, (90, 250)
23	*143*, (75, 248)

creased either more rapidly or more slowly than this, the results are less satisfactory.

Using animals optimally pretreated in this manner, we were able to show in a large number of experiments that in the stage of resistance, the stimulus to which adaptation occurred produces little or no thymus involution, while other stimuli may cause an even more marked involution in these resistant animals than they do in not pretreated controls. We shall not report on all the experiments of this series but we wish to describe one as an example.

The experiment was so planned that all the animals were three months old at the end of the pretreatment, that is, at the time when they received the full alarming dose of the stimulus for which they had been adapted. The full alarming dose as defined above, for the drugs used here and for rats 3–4 months of age is: 0.2 cc. of 1 per mille solution of adrenaline hydrochloride; 0.5 cc. of a 4 per cent solution of formaldehyde; 2 cc. of a 1

per cent solution of morphine sulphate and 2 cc. of 1 per cent solution of atropine sulphate given subcutaneously twice daily on two consecutive days.

For the sake of uniformity only female rats were used in this series, although other experiments (not reported here) show that adaptation occurs in males in a very similar manner. Each group consisted of 24 rats. One group had been adapted to morphine, one to adrenaline and one to atropine. In all these groups, one quarter of the alarming dose of the respective drug was given to start with, and this amount was gradually raised to the full alarming dose within 10 days. A fourth group had been adapted to muscular exercise, starting with 10 minutes twice daily in revolving cages with a diameter of 12 inches and running at a speed of 18 to 22 revolutions per minute. The amount of exercise was increased daily, so that at the end of three weeks, the animals were able to run one and one-half hours twice daily without any difficulty. Previous experiments had shown that this is the full alarming dose for exercise. In this group it proved necessary to give a somewhat longer pretreatment than in the others. The fifth group had been adapted to cold by gradually decreasing the surrounding temperature to −4°C. within 10 days. Two days spent at a temperature of about −4°C. represents the full alarming dose for cold. As soon as the full alarming dose of these five stimuli had been reached, each series was subdivided into groups of 6 animals. One of these groups was exposed to the full alarming dose of the stimulus to which they had been adapted and the other three to a stimulus of a different nature. At the same time, a group of 6 not pretreated control animals was exposed to the full alarming dose of each of these stimuli. In the atropine pretreated series, six animals died during pretreatment so that only three groups were left for the final test. It is evident from the data summarized in table 2 that pretreatment with a certain alarming stimulus increases the resistance to this particular stimulus above that of not pretreated animals, but simultaneously decreases the resistance to alarming stimuli of a different type. This was especially obvious in the case of inurement to more physiological stimuli, such as muscular exercise or cold, to which more complete and more lasting habituation is obtained than to drugs.

In order to avoid the skin lesions which usually result from chronic subcutaneous administration of atropine, the drug was given intraperitoneally in this experiment in which prolonged pretreatment was necessary. For the sake of uniformity this method of administration was also used in the groups receiving atropine after pretreatment with another drug. While the atropine pretreated animals stood these daily intraperitoneal injections of atropine without ill effects, it became evident during the experiments that this same dose of atropine given intraperitoneally is fatal

for not pretreated animals. However the not pretreated animals survived this dose longer than the animals adapted to other stimuli, while there

TABLE 2

Showing that while adaptation is acquired to a certain stimulus the resistance of rats to other stimuli decreases

PRETREATED WITH	EXPOSED TO FULL ALARMING DOSE OF	THYMUS WEIGHT	NUMBER OF CASUALTIES
		mgm.	
Not pretreated	Not treated	*245* (178, 294)	0
	Adrenaline	*127* (107, 205)	0
	Atropine*	*124* (72, 285)	0
	Atropine†	All died within 24 hours	
	Morphine	*113* (89, 154)	0
	Cold	*125* (115, 145)	0
	Muscular exercise	*114* (82, 155)	0
Adrenaline	Adrenaline	*174* (90, 250)	0
	Atropine†	All died within 12 hours	
	Morphine	*71* (65, 79)	0
	Muscular exercise	*59* (44, 60)	0
Atropine	Atropine†	*60* (45, 75)	0
	Morphine	*40* (34, 50)	3
	Cold	*42* (21, 72)	2
Morphine	Adrenaline	*64* (47, 94)	0
	Atropine†	All died within 12 hours	
	Morphine	*118* (83, 156)	0
	Cold	All died within 24 hours	
Cold	Adrenaline	*135* (101, 159)	1
	Morphine	*183* (166, 200)	2
	Cold	*225* (183, 325)	0
	Muscular exercise	*93* (74, 125)	1
Muscular exercise	Adrenaline	*107* (80, 148)	0
	Atropine*	*75* (48, 91)	1
	Cold	*199* (168, 211)	2
	Muscular exercise	*252* (191, 285)	0

* Subcutaneously.
† Intraperitoneally.

were no casualties at all in the group which had been previously adapted to this drug. Thus this experiment also supports the conception of the specificity of adaptation although the thymus weights gave us no information

regarding this point since many of the animals died before thymus atrophy could have occurred. It appears from the data of this table that habituation even to such physiological stimuli as muscular exercise or cold is acquired at some expense—namely, at the expense of adaptation energy—so that animals adapted to such stimuli lose much of their natural innate ability to adapt themselves to a stimulus of a different type.

In order to obtain more explicit proof that such specific adaptation is connected with a decrease in the ability of animals to survive lethal doses of other drugs, another series of experiments was performed in which the number of survivals in a certain group was taken as an index of resistance instead of the thymus weight.

Twelve female rats, three and one-half months old, were pretreated with ascending doses of morphine sulphate, beginning with 2 cc. of a 1 per cent solution subcutaneously twice daily, the dose being gradually increased within 19 days to 2 cc. of a 3 per cent solution 3 times daily. The animals were then divided into two groups. In 6 rats treatment with the same dose of morphine was continued, while the remaining 6 received a single injection of 0.25 cc. of a 2.5 per cent solution of alpha dinitrophenol, in Mazola oil, subcutaneously; the same dose of dinitrophenol was also administered to 6 not pretreated controls. All the morphine pretreated animals died within two and one-half hours after the injection of the dinitrophenol, while only one of the not pretreated controls succumbed during this time. (They all succumbed after two more injections of the same magnitude given during the same day.) The resistance to dinitrophenol was decreased, therefore, by the morphine pretreatment. That the resistance towards morphine was actually increased above normal in the dinitrophenol sensitive rats is shown by the fact that the remaining 6 tolerated 2 cc. of a 3 per cent morphine solution 3 times daily without casualties or even very marked symptoms of morphine intoxication, while 3 out of 6 not pretreated controls which received this dose of morphine daily died within four days. Autopsy disclosed typical symptoms of the alarm reaction. The results of this experiment are summarized in table 3 (expt. I).

That resistance to dinitrophenol may also be acquired was shown in another group of 6 rats of the same age and sex receiving ascending daily doses of this drug for 8 days. At the end of this period they tolerated 0.25 cc. of the 2.5 per cent solution 4 times a day for 4 consecutive days without casualties. This shows a definite increase in the resistance as compared with the not pretreated controls mentioned above, of which none tolerated even as much as 3 injections given in one day (table 3, expt. 2).

In another series, two months old male rats were used. Twelve animals were adapted to atropine (15 days' pretreatment with daily doses as-

cending from 1 cc. of the 2 per cent solution to 2 cc. twice daily intraperitoneally) and 18 to adrenaline (15 days' pretreatment with daily doses ascending from 0.2 cc. of 1 per mille solution subcutaneously to 0.3 cc., 3 times daily). Six adrenaline pretreated animals were treated with 0.3 cc. of a 1 per mille solution of adrenaline 3 times daily, together with 6 not pretreated animals. While all the adrenaline pretreated animals survived, 3 of those not pretreated succumbed within 96 hours. After the acquisition of adaption to adrenaline was thus ascertained, the remaining 6 adrenaline adapted animals were treated with 2 cc. of a 2 per cent solution of atropine twice daily, together with 6 atropine pretreated and 6 not pretreated controls. All adrenaline pretreated animals succumbed within

TABLE 3

Showing that while adaptation is acquired to a certain stimulus the resistance of rats to other stimuli decreases

NUMBER OF EXPERIMENT	PRETREATMENT	TREATMENT	NUMBER OF CASUALTIES IN GROUP OF 6
1	Morphine	Dinitrophenol*	6
	Morphine	Morphine	0
	Not pretreated	Dinitrophenol	1
	Not pretreated	Morphine	3
2	Dinitrophenol	Dinitrophenol	0
	Not pretreated	Dinitrophenol	6
3	Adrenaline	Adrenaline	0
	Not pretreated	Adrenaline	3
	Adrenaline	Atropine	6
	Atropine	Atropine	0
	Not pretreated	Atropine	2
	Adrenaline	Formaldehyde	6
	Not pretreated	Formaldehyde	1

* The dose of dinitrophenol used in experiment 1 is smaller than that employed in experiment 2 (see text).

32 hours after the first injection, while only 2 of the not pretreated and none of the atropine pretreated succumbed during this time.

The remaining 6 adrenaline pretreated rats received 0.5 cc. of a 4 per cent solution of formaldehyde subcutaneously 3 times daily, together with 6 not pretreated controls of the same age and sex. While all the adrenaline pretreated animals died within 35 hours after the first injection, only one of the not pretreated rats succumbed during this time (table 3, expt. 3).

All these experiments corroborate the conception that the adaptation acquired in the stage of resistance is specific and that while adaptation occurs to a certain drug the organism exhausts its abilities to adapt itself to others. That means that adaptation is always acquired at a cost.

SUMMARY AND CONCLUSIONS

The experiments reported in this communication show that by giving gradually increasing doses of various alarming stimuli, one may raise the resistance of animals in such a manner that even treatment which would cause marked thymus involution in normals will have little or no effect on the thymus weight. On the other hand, stimuli other than the one with which these animals had been pretreated will cause severe thymus involution which is usually even more pronounced than that obtained by the same dose of the same agent in the not pretreated animal. Using the response of the thymus as an index of resistance, one may say, therefore, that during adaptation to a certain stimulus the resistance to other stimuli decreases.

This conception receives further support by experiments showing that rats pretreated with a certain agent will resist such doses of this agent which would be fatal for not pretreated controls. At the same time, their resistance to toxic doses of agents other than the one to which they have been adapted decreases below the initial value.

These findings are tentatively interpreted by the assumption that the resistance of the organism to various damaging stimuli is dependent on its adaptability. This adaptability is conceived to depend upon adaptation energy of which the organism possesses only a limited amount, so that if it is used for adaptation to a certain stimulus, the resistance to other stimuli will necessarily decrease. We conclude that adaptation to any stimulus is always acquired at a cost, namely, at the cost of adaptation energy.

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RESPIRATORY EFFECTS FROM THE APPLICATION OF COCAINE, NICOTINE, AND LOBELINE TO THE FLOOR OF THE FOURTH VENTRICLE[1, 2]

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Received for publication June 15, 1938

In a previous series of experiments one of us (Nicholson, 1936) studied in dogs the effects upon respiration of cooling the floor of the fourth ventricle. It was found that while severe cooling might completely stop breathing, the typical effect of local cooling of the obex region was an alteration in the relative durations of the inspiratory and expiratory phases of respiration in favor of the former. This effect might vary in degree from a slight decrease in the duration of the expiratory pause to true apneustic breathing in which the duration of inspiration is extremely prolonged. These results seemed to remove the necessity for the existence of a separate "pneumotaxic" center (Lumsden, 1923) and suggested that inspiration is normally interrupted and expiration initiated through the activity of some mechanism located near the floor of the fourth ventricle at about the level of the calamus scriptorius. Since very slight lowering of the temperature of the floor of the fourth ventricle was sufficient to produce definite respiratory changes, this expiratory mechanism appeared to be very superficially placed.

In view of these results showing the ease with which a portion of the central respiratory mechanism could be depressed by superficial cooling it seemed worth while to investigate the effects of drugs applied to the floor of the fourth ventricle, particularly those drugs which might also be expected to depress nervous function.

The first drug used was cocaine. In all these experiments dogs, anesthetized with either morphine and urethane or pentobarbital were used. In the early experiments a few drops of a one or two per cent solution of cocaine in Locke's solution or a small piece of filter paper moistened in such a cocaine solution were placed on the floor of the fourth ventricle in the calamus region. Since, however, the surface of the brain stem is normally bathed with cerebrospinal fluid, it was found that even though

[1] Preliminary Report: This Journal, Proc. **116:** 114, 1936 and Proc. **119:** 380, 1937.

[2] These experiments were supported in part by a grant from the Rockefeller Foundation to Robert Gesell for studies on respiration.

the cerebrospinal fluid was removed by aspiration immediately before applying the cocaine, the cocaine was quickly washed away or diluted. In later experiments this difficulty was to a large extent eliminated and a gradual absorption of larger amounts of cocaine obtained by spreading on the surface of the obex a cocaine ointment made by mixing one part of cocaine with about three parts of vaseline.

The application of cocaine in solution to the obex in a few cases resulted promptly in apnea and death unless artificial respiration was immediately instituted. However, when apnea did not occur, the respiratory effects, though in many cases profound, were invariably quite transient. Figures 1, A, B, and C show typical effects of cocaine applied in this way. In

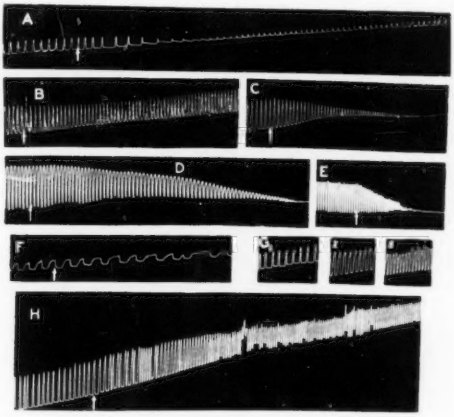

Fig. 1. Effects on respiration of the application of cocaine to the floor of the fourth ventricle. D and E are records of circumference changes of the abdomen, downstroke representing inspiration. Other records are spirometer tracings, upstroke representing inspiration. In A, B, and C the cocaine was applied in solution. In D, E, F, G, and H it was mixed in vaseline. Arrows indicate moment of application of the cocaine.

figure 1 A, 0.04 cc. 2 per cent cocaine produced, after a short latent period, a marked depression of respiration evidenced by a decrease in respiratory rate from 11.5 per minute to 7 per minute and a decrease in the amplitude of the spirometer tracing from 10 mm. to 1.5 mm. There soon followed, however, a marked increase in respiratory rate and a gradual increase in amplitude until in a few minutes the respiration had returned to normal. In figure 1 B, 0.1 cc. of 1 per cent cocaine produced only a slight decrease in amplitude of respiration with a comparable increase in rate, both effects soon passing off. In figure 1 C, from the same animal as 1 B, 0.1 cc. 2 per cent cocaine caused the amplitude of respiration rapidly to decrease to zero, the decrease in amplitude being accompanied by an increase

in rate. Death apparently would have occurred if artificial respiration had not been initiated.

Figure 1 D, E, F, G, and H show typical effects of cocaine applied to the surface of the medulla in vaseline. In figure 1 F, the principal effect of cocaine was a marked increase in the duration of inspiration so that true apneustic breathing resulted quite similar to that seen in the experiments on local cooling of the floor of the fourth ventricle. In these experiments on cooling it was found that if the temperature was lowered only slightly the only effect observed might be a slight decrease in the duration of the expiratory pause. As the temperature was further lowered, the expiratory pause was shortened still more while the duration of inspiration was gradually increased until real apneusis resulted with marked respiratory slowing. If the temperature was lowered to the point at which the expiratory pause was markedly shortened, but not low enough to result in a marked prolongation of inspiration, there might be a considerable acceleration of respiration, while if the duration of inspiration was increased as much as the duration of expiration was decreased, the respiratory rate might be unchanged. These various combinations of shortening of the expiratory phase and lengthening of the inspiratory phase of respiration have also been observed following the application of cocaine, two examples of which are seen in figures 1 G and H. G_1 is a normal record. G_2 shows the effect of an application of cocaine in vaseline, a definite shortening of the expiratory pause with a slight prolongation of inspiration, the resultant effect on respiratory rate being a 40 per cent increase. Figure 1 G_3 shows the effect of a subsequent application of the same amount of cocaine. The expiratory pause has now been completely abolished and although there is a definite inspiratory pause present, giving the respiration a slightly apneustic appearance this prolongation of inspiration is much less than the shortening of expiration so that the final effect is an increase in rate of 120 per cent compared with G_1. In figure 1 H cocaine completely abolished the expiratory pause with only a very slight increase in the duration of inspiration, resulting in an increase in respiratory rate of 132 per cent. Records 1 G and H are completely indistinguishable from similar records obtained by local cooling of the obex region.

When central cooling was carried to the point at which cessation of respiration was brought about, reappearance of respiration on discontinuance of the cooling was introduced by a period of increasing inspiratory tonus with no sign of expiratory activity, suggesting that the inspiratory mechanism which was less readily depressed than the expiratory also recovered more rapidly. A somewhat similar increase in inspiratory tonus may be seen following the application of cocaine. In figures 1 D and E it shows up quite clearly, suggesting that the expiratory mechanism may be depressed somewhat more readily by cocaine than the inspiratory.

Since it appeared that the effects on respiration of cocainization of the medulla were quite similar to the effects of cooling, it seemed worth while to investigate the action of cocaine upon the effectiveness of various procedures which ordinarily markedly alter respiration, as was done in the case of cooling by Nicholson and Brezin (1937). In this connection we studied the effects of inflation and deflation of the lungs, of faradic stimulation of the central end of the vagus nerve, of faradic stimulation of a somatic sensory nerve, the saphenous, and of intratracheal administration of carbon dioxide.

Inflation and deflation of the lungs were accomplished by increasing the weight of the mercury buoyed spirometer, and of its counterweight respectively. Normally, inflation (fig. 2 A) decreases respiratory rate while deflation (fig. 2 B) increases it. If one compares figures 2 C and D with 2 A and B, respectively, he sees that following cocainization of the region of the calamus scriptorius these effects are almost or completely

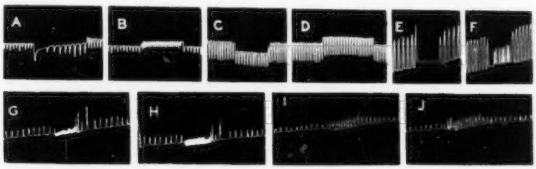

Fig. 2. Alteration by cocaine of the respiratory effects of inflation and deflation of the lungs, central vagal stimulation and saphenous stimulation. A, B, C, and D are records of circumference changes of the abdomen, downstroke representing inspiration. Other records are spirometer tracings, upstroke representing inspiration.

abolished. Similarly the effects on respiration of central vagal stimulation are markedly depressed or abolished by cocainization of this region. In figure 2 E is seen complete cessation of respiration resulting from stimulation of the central end of the vagus while as seen in 2 F the same stimulus after cocainization caused a very brief pause followed by acceleration. These effects of cocaine on vagal respiratory reflexes are similar to the effects of severe cooling.

However, when we compare the alteration in the respiratory effects of saphenous nerve stimulation and carbon dioxide administration by cocaine with the effects of cooling upon these procedures we see a marked difference. Whereas the accelerating effect of saphenous stimulation was enhanced by local cooling of the obex region, it was markedly diminished by cocainization of this area. In figures 2 G and H we find two strengths of stimulation producing increases in respiratory rates of 1014 per cent and 740 per cent respectively. In figures 2 I and J we see the same stimuli, after cocaine, increasing the respiratory rate 87 per cent and 59 per cent.

It was previously found that the accelerating effect of carbon dioxide upon respiration in some cases was greatly exaggerated by local cooling of the floor of the fourth ventricle. Local application of cocaine to the calamus region never had this effect. Sometimes the effect of carbon dioxide was unaltered by cocaine, but in most cases, and this was particularly true when the normal response to carbon dioxide was a considerable increase in rate, the response was diminished by cocaine. In a typical experiment a 12 per cent carbon dioxide mixture, before the application of cocaine, increased the respiratory rate 50 per cent while after cocaine administration, the same mixture caused only a 12 per cent increase. These differences between the effects of cocaine and cooling are not readily explained, but are probably related to the difficulty of regulating both the intensity and the extent of the effects of cocaine as compared with the relative ease with which these factors can be controlled in the case of cooling.

We also studied the effects of the application of nicotine in Locke's solution to the floor of the fourth ventricle. It has been generally found that nicotine intravenously in small doses stimulates respiration, in larger doses paralyzes it. Heymans, Bouckaert, and Dautrebande (1931) and Mercier and his co-workers (Mercier, Rizzo and Delphaut, 1934; Mercier and Delphaut, 1936) have both reported that the stimulant action is exerted largely through the carotid gland region, though both groups agree that central stimulation may also occur, the stimulation being usually preceded by inhibition. It is commonly stated that nicotine acting on nervous tissue in general first stimulates, then paralyzes.

We found that the application of nicotine, 0.5 per cent to 5 per cent, to the obex region invariably produced a cessation of respiration with the chest in the expiratory position. The latent period of the effect was apparently zero. Figures 3 A to F show a number of examples of the effect of nicotine applied in this way. In addition to the respiratory effects there occurred an immediate slowing of the heart and drop in blood pressure. The exceedingly rapid action of nicotine contrasted with the slowness with which cocaine produces apnea suggests that the two drugs are acting in entirely different ways. Apnea from cocaine is certainly the result of depression of the respiratory center or centers. In contrast the instantaneous apnea produced by nicotine is probably the result of stimulation of some expiratory mechanism. This stimulation may consist either in the direct excitation of an expiratory "center" or the facilitation of afferent impulses which tend to bring about expiration. That this apnea is due to stimulation and not depression is further supported by the sharp decrease in pulse rate, probably the result of stimulation of the cardio-inhibitory center. Evidence from the cooling experiments for the existence of an expiratory mechanism located very near the surface in this region lends further support to this view.

If this belief in regard to the nature of nicotine apnea is correct, we should find that the effect of nicotine on the duration of the various phases of the respiratory cycle should be just the opposite of cocaine and cooling. These latter procedures tend to produce a slow, prolonged inspiration—an apneustic type of breathing. If we examine the respirations immediately following the nicotine apnea or as in figure 3 C, the respirations during the initial nicotine effect in which the slowing of respiration is not sufficient to be considered apnea, we find that inspiration is actually more rapid and terminates more suddenly than normally. That is, the respiration approaches the gasping type, just the opposite of apneusis. This is well shown in figure 3 A in which the normal respiration showed some tendency toward apneusis.

This contrast between the action of nicotine and that of cocaine and cooling is again evident when one examines the effect of nicotine upon

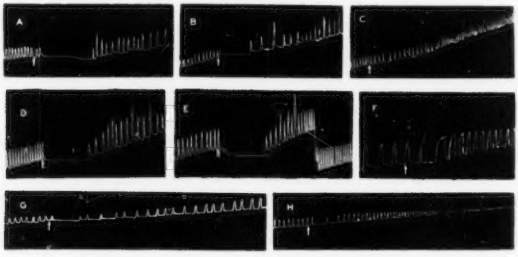

Fig. 3. Respiratory effects from the application of nicotine (A, B, C, D, E, F) and lobeline (G, H) to the floor of the fourth ventricle. The records are spirometer tracings, upstroke representing inspiration. Arrows indicate moment of application of the drug.

the tonus of the respiratory muscles. As we have already pointed out apnea produced by cocaine or severe cooling is associated with an increase in tonus of the inspiratory muscles, due apparently to depression of an expiratory mechanism. If, however, we examine figures 3 A, D, and E, we see that nicotine apnea is associated with increasing *expiratory* tonus probably due to *stimulation* of an expiratory mechanism.

One further observation might be mentioned in this connection; viz., that it was found impossible to interrupt the nicotine apnea by stimulation of the saphenous nerve while during apneustic breathing produced by cooling, such sensory stimulation was even more effective than normally in accelerating respiration.

Since nicotine is believed first to stimulate and then to paralyze nervous tissues, the possibility of later effects due to depression should be considered. It was observed that frequently with the use of 5 per cent nico-

tine and occasionally with the use of lower concentrations, there occurred an acceleration of respiration following the initial apnea. This acceleration appears to be purely the result of a decrease in the duration of the expiratory pause, in this respect resembling the acceleration produced by cocaine or by moderate cooling and it may be that it represents the effect of a moderate depression of the expiratory mechanism. This belief is supported by the long duration of the acceleration, frequently two or three hours, and by the fact that during the acceleration it is difficult or impossible again to produce apnea by a second application of nicotine. Figure 3 C shows the effect of application of 0.03 cc. 5 per cent nicotine some time after the application of 0.05 cc. of 1 per cent nicotine shown in figure 3 B. As can be seen, in spite of the fact that the second dose was much greater than the first, the slowing effect was much less. In other experiments in which 5 per cent nicotine was used initially, a second application produced no slowing whatever.

Figure 3 E shows the effect of nicotine after denervation of both carotid gland regions. Figure 3 D is a nicotine effect in the same animal before denervation. Obviously the effect after nicotine is perfectly typical of nicotine effects in intact animals. Of course, the exceedingly brief latent period of the nicotine apnea makes it exceedingly improbable that any extra-medullary factors are involved.

Figure 3 F shows the effect of nicotine after section of both vagi. Again the respiratory effects are essentially those seen in the intact animal. It is of interest that the drop in blood pressure and slowing of the heart still occur, though the latter is somewhat less marked than usually seen in animals with intact vagi.

Since alpha-lobeline had been advertised as the specific stimulant to the respiratory center we studied also the effects of its application directly to the floor of the fourth ventricle. It is generally agreed that, as originally pointed out by Edmunds (1904), the pharmacological actions of lobeline are essentially the same as those of nicotine. As regards respiration it stimulates in small doses and paralyzes in large. Heymans, Bouckaert and Dautrebande (1931) localized the stimulating action of lobeline on respiration to the carotid gland region, though Clementi (1928) reported stimulation of respiration from application of lobeline to the floor of the fourth ventricle. Our results support the view that its respiratory effects are essentially those of nicotine, although we never observed a well marked secondary augmentation of respiration as sometimes occurred with nicotine. Typical effects are seen in figures 3 G and H. There is ordinarily an immediate apnea which, as in the case of nicotine, we are inclined to regard as due to stimulation of an expiratory mechanism. In many cases, as in 3 H, recovery from the apnea quickly gives way to a progressive decrease in respiratory volume which may extend to apnea and death.

This secondary respiratory depression we regard as the result of true depression and paralysis of the respiratory center. In several experiments we applied lobeline in progressively increasing concentrations beginning with a concentration too low to have any effect and never did we observe respiratory stimulation.

These results then appear to support the view that, as regards respiration, the central action of lobeline is predominantly depressant, and the respiratory stimulation which may result from its intravenous injection is largely the result of its action at the carotid gland. The recent experiments of Snyder and Rosenfeld (1937) strongly suggest that at least in rabbits the carotid gland is non-functional at birth. If this is true also of humans, it would appear that the use of lobeline as a respiratory stimulant might be an even more uncertain procedure in the newborn than in the adult.

SUMMARY

The respiratory effects on anesthetized dogs of cocaine, nicotine, and lobeline applied to the floor of the fourth ventricle in the obex region were studied and compared with the effects of cooling that region.

The effects of cocaine duplicated to a very marked extent the effects of cooling. If applied in sufficient concentration, apnea and death may result, the apnea being frequently preceded by a period of increasing inspiratory tonus. If the absorption of the cocaine is not too rapid, true apneustic respiration with decided prolongation of inspiration may occur. The various stages leading up to apneustic breathing observed in the cooling experiments have also been observed following the application of cocaine. It appears that cocaine applied to the floor of the fourth ventricle is able to paralyze the entire central respiratory mechanism, but that the expiratory mechanism is more readily depressed than the inspiratory.

Central application of cocaine depresses or abolishes the respiratory effects of inflation and deflation of the lungs, of electrical stimulation of the central end of the vagus, of saphenous nerve stimulation and of intratracheal administration of CO_2.

Nicotine causes an instantaneous apnea, the result, we believe, of stimulation of an expiratory mechanism. This apnea is sometimes followed by a prolonged acceleration of breathing similar to that resulting from moderate cooling and due, we believe, to the same cause—moderate depression of the expiratory mechanism.

The effects of lobeline were similar to those of nicotine except that definite augmentation of respiration was never observed.

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BALTIMORE, U. S. A.

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Entered as second-class matter, August 18, 1914, at the Post Office in Baltimore, Md., under the act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in section 1103, Act of October 3, 1917. Authorized on July 5, 1918

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HISTORY OF THE AMERICAN PHYSIOLOGICAL SOCIETY

To commemorate the Semicentennial of its foundation the American Physiological Society has published an illustrated history, with index, of its development since 1887. The book contains articles by William H. Howell and Charles W. Greene, with a foreword by Walter J. Meek, and was distributed in May, 1938.

The book of about 220 pages is in case-binding and costs $2.50.

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